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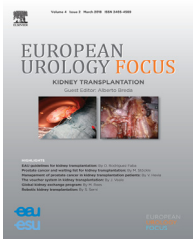

Bacteriophages: a Panacea in Neuro-Urology?

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Abstract

Bacterial urinary tract infections (UTIs) are very frequent, especially in patients with neurogenic lower urinary tract dysfunction (NLUTD). The steady increase in antibiotic resistance among causative bacteria prompts the search for highly effective therapeutic alternatives with little or no side effects. Bacteriophages — obligate intracellular viruses that solely infect and kill bacteria — are promising tools for treating bacterial infections and have been used for this purpose for almost a century. Recent clinical studies using bacteriophage therapy for UTIs showed encouraging results. In particular, patients with recurrent UTIs, such as individuals with NLUTD who rely on assisted bladder emptying, might benefit from this treatment method. However, bacteriophages are not yet a panacea. More high-quality basic and clinical research on bacteriophage therapy is needed to answer questions on the use of this therapeutic option and its potential to provide a solution to the global threat of multidrug-resistant bacteria.

Patient summary: Urinary tract infections are very common, especially in patients with neurogenic lower urinary tract dysfunction. In this review we discuss the potential of bacteriophage therapy as an alternative to antibiotics for treating patients with bladder infections.

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Urinary tract infections and the threat of antibiotic resistance

Community- and hospital-acquired urinary tract infections (UTIs) are among the most common diagnoses in clinical practice [1] and account for a substantial proportion of all antibiotics prescribed worldwide. Patients with neurogenic lower urinary tract dysfunction (LUTD) have a higher probability of UTIs as a consequence of multiple risk factors, such as assisted bladder emptying, urine stasis, high bladder pressure, and bladder stones [2]. Among patients with a spinal cord injury, the average UTI rate is approximately 2.5 per year [2]. However, especially in these patients, UTI diagnosis is challenging and the high rate of asymptomatic bacteriuria can lead to uncritical use of antibiotics. Misuse of antibiotics is regarded as the main driver of antibiotic resistance, a rapidly emerging major threat to public health. Substantial resistance rates to almost all antibiotics are present in most UTI pathogens, with broad geographic variations [1]. Reports of bacteria resistant to more than 20 commonly used antibiotics have emerged. The situation is worsened by recent warnings on fluoroquinolone use from medical authorities worldwide (European Medicines Agency, www.ema.europa.eu/en/medicines/human/referrals/quinolone-fluoroquinolone-containing-medicinal-products), further limiting treatment alternatives. Novel, well-tolerated therapeutic options for treating UTIs and reducing antimicrobial resistance are urgently needed.

Bacteriophages: a short overview

The word *bacteriophage* is derived from the Greek words *βακτήριον* (baktérion) and *φαγεῖν* (phageín) meaning “to devour rods” or “bacteria eater” (Fig. 1). Bacteriophages are obligate intracellular viruses that infect bacteria and can have different life cycles (Fig. 2). They are the world’s most

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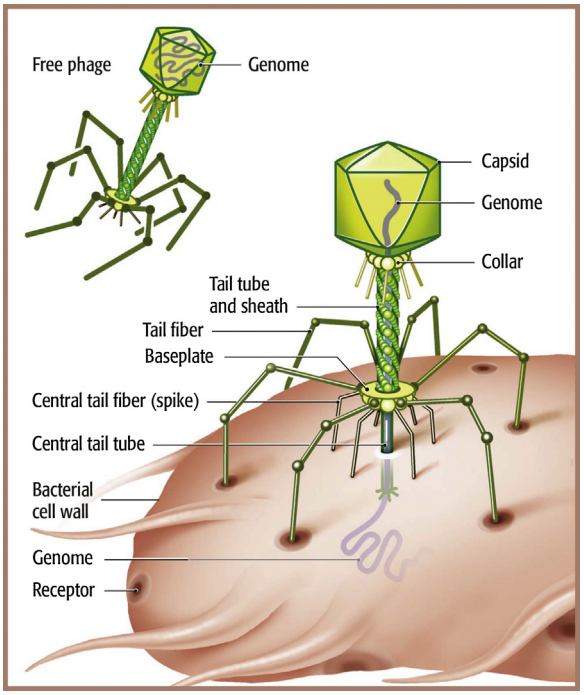

Fig. 1 – Bacteriophage morphology and action. A free bacteriophage targets a susceptible bacterial host and attaches to the bacterial cell wall by binding with the tail fibers to surface receptors. The bacteriophage infects the bacterium, injecting its genome via the central tail tube. The surface receptors are usually very specific, so a particular bacteriophage can only infect a narrow range of bacteria.

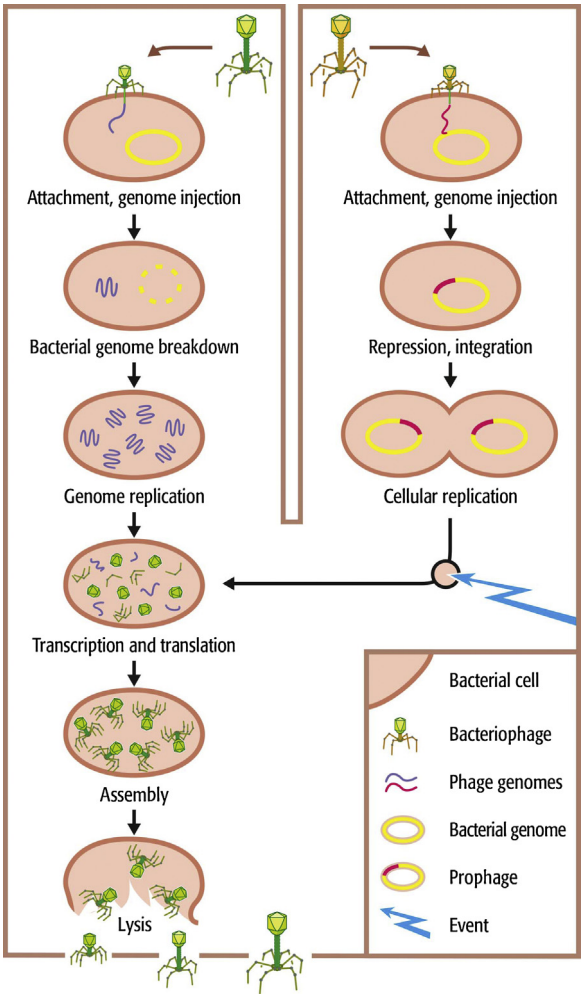

Fig. 2 – Lytic and temperate (or lysogenic) bacteriophage cycles. A bacteriophage infects a susceptible bacterial host via genome injection. In the lytic pathway (left), the bacterial genome breaks down and the bacteriophage genome replicates, resulting in mature new bacteriophage clones that burst the bacterial host and diffuse through the surrounding environment, were they can infect new susceptible bacteria. Temperate (or lysogenic) bacteriophages (right) have a different strategy. After injection of the genome, it becomes integrated into a specific section of the bacterial genome and will be replicated every time the bacterial cell duplicates. The bacteriophage genome integrated in the bacterial genome or existing as an extrachromosomal plasmid is called a prophage. Cell divisions produce a population of bacterial cells harboring the bacteriophage genomic material. Environmental factors (such as starvation or other unfavorable growth conditions) can induce a temperate (or lysogenic) bacteriophage to enter the lytic cycle (left). Bacteriophage therapy exclusively uses lytic bacteriophages to treat pathogenic bacterial infections.

abundant biological entities and play a major role in almost all ecosystems and in bacterial evolution [3]. Bacteriophages are the major driver of horizontal gene exchange between bacteria.

Frederick Twort and Felix d'Hérelle discovered bacteriophages independently of each other approximately one century ago. Research on bacteriophages has revolutionized our understanding of molecular biology and biotechnology, with milestones such as the discovery of restriction enzymes, genome sequencing, genetic control mechanisms, and the development of genome engineering; an example is the promising CRISPR-Cas method, originally a bacteriophage-resistance system discovered in bacteria [3].

The literature also contains evidence of bacteriophages as obligate members of the human microbiome with a modulatory effect on the bacterial composition in the gut and on the immune system. It has also been shown that bacteriophages are part of the innate immune system in the mammalian gut [4]. Recent research indicates that bacteriophages contribute to bladder health and have an influence on the pathophysiology of conditions such as overactive bladder syndrome and UTIs.

Early bacteriophage research in the 1920s and 1930s focused on the development of bacteriophage therapy to treat bacterial infections. The discovery of penicillin by Alexander Fleming and subsequent mass production in the 1940s led to a rapid decline in interest in bacteriophage therapy in the Western world. However, research continued in the former Soviet Union and other Eastern European countries, where bacteriophage therapy is currently well accepted and an integral part of several health care systems. The growing threat of antibiotic resistance has prompted renewed interest in bacteriophages as therapeutic alternatives to antibiotics in the Western world.

Bacteriophage therapy for UTIs: current situation

Bacteriophage therapy consists of the treatment of particular bacterial pathogens using natural or modified virulent, obligate lytic bacteriophages (Fig. 2). Despite a long history of use in Eastern Europe and several successful applications under modern clinical standards, high-level evidence from research compliant with modern clinical standards on bacteriophage therapy is scarce.

Several considerations are needed when selecting the model disease and the bacteriophage for a therapeutic approach [5,6] and UTIs are an ideal clinical model for further research. Owing to its constitution as an almost "closed compartment", the urinary bladder is of outstanding interest as highly amenable for bacteriophage instillation treatments, which allow excellent interaction between the bacteriophage and the bacterial pathogen in this circumscribed body cavity. Especially in patients with neurogenic LUTD, who often rely on catheterization for urine evacuation, a therapeutic bacteriophage solution could easily be instilled and used for acute, recurrent, or chronic UTIs. This treatment approach might reduce the overuse of antibiotics and protect these patients against multidrug-resistant bacteria, ultimately resulting in an optimized treatment strategy and promotion of bacteriophage therapy.

First successful examples of bacteriophage therapy in urology have been published. A considerable reduction in biofilm formation was found after "coating" indwelling catheters with bacteriophages specific for *Pseudomonas aeruginosa*, *Escherichia coli*, and *Proteus mirabilis* [7]. Khawaldeh et al. [8] reported success with adjunctive bacteriophage therapy for refractory *P. aeruginosa* UTI in a patient with repeated failure of antibiotics. The combined therapy was well tolerated and resulted in symptom relief and microbiological cure.

In a project between Switzerland (Department of Neuro-Urology, Balgrist University Hospital, University of Zürich) and Georgia (Alexander Tsulukidze National Center of Urology and Eliava Institute of Bacteriophage, Microbiology and Virology, Tbilisi), *in vitro* experiments showed up to 93% lytic activity for commercially available bacteriophage cocktails (Pyo bacteriophage and Enko bacteriophage) on 41 *E. coli* and nine *Klebsiella pneumoniae* strains of isolates from urine of patients with neurogenic LUTD suffering from acute UTIs [9]. A subsequent *in vivo* study [10] revealed a decrease in bacterial titer (between 1 and 5 log) in 67% of participants after 7-d intravesical bacteriophage instillation therapy. No bacteriophage-related adverse events were reported. These studies led to the world's first randomized controlled trial of bacteriophages for treating UTIs (ClinicalTrials.gov NCT03140085) [11].

Hurdles for the implementation of bacteriophage therapy in daily clinical practice

Despite many exciting developments, the use and acceptance of bacteriophage therapy in urology in the Western world are still in their infancy. At present, regulatory rules do not allow the use of bacteriophage therapy in urology outside of clinical studies, or only as an individualized experimental therapeutic attempt. Even if the long-lasting effect of bacteriophage therapy is confirmed as safe, the therapeutic value needs further confirmation and previous studies do not meet all the requirements of modern evidence-based medicine [5]. Many critical factors and important questions need to be addressed before possible market availability, beginning with the choice of therapeutic bacteriophage(s), cocktail composition, production settings, and purification of bacteriophage lysates. However, bacteriophage therapy has the potential to solve the current antibiotic crisis in the near future if applied competently and carefully. Engineered bacteriophages (Fig. 3) [12] might completely revolutionize the field, considering the success in a 15-yr-old patient with life-threatening disseminated antibiotic-resistant *Mycobacterium abscessus* infection with the first genetically engineered bacteriophage treatment [13].

Conclusions

Bacteriophage therapy is a very promising alternative to antibiotics in the treatment of bacterial infections and has the potential to counteract resistant bacteria. More clinical results achieved with bacteriophage therapy will increase awareness of this potentially revolutionizing treatment option among the broader public. However, rigorous clinical

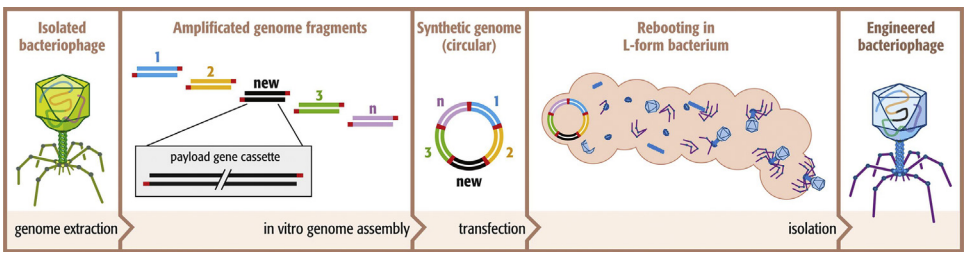

Fig. 3 – Bacteriophage engineering methods and strategies. Workflow for construction of engineered bacteriophages. The bacteriophage genome is extracted and divided into fragments. Synthetic genomes are assembled from large genome fragments containing the desired payload gene cassette (indicated as a black fragment) and subsequently transformed into a suitable surrogate host for genome rebooting. After transcription and assembly, the newly engineered bacteriophages with the desired functions can be isolated [12].

studies including optimal patient populations such as those with neurogenic LUTD are needed to prove the concept of bacteriophage therapy and to make it a panacea in neuro-urology.

Conflicts of interest

The authors have nothing to disclose.

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