PHAGE THERAPY AS AN ALTERNATIVE TO ANTIBIOTIC THERAPY AGAINST URINARY TRACT INFECTIONS TO COMBAT ANTIBIOTIC RESISTANCE

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Abstract

Urinary tract infection is the global health problem being 2nd most common bacterial infection. The abuse of antibiotics without bacterial characterization during its treatment results in emergence of highly antibiotic-resistant bacteria. Some biofilm forming bacterial strains are hard to treat using antibiotics. So, an alternative therapy is urgently needed. Phage therapy offers promising results for treatment of these infections in which virulent or lytic phages are used to lyse infection-causing bacterial cells. This review emphasizes several ways in which phage therapy can be carried out against uropathogenic bacteria. Clinical trials provide many positive results for phage therapy as compared to antibiotic therapy but a lot of in-vivo studies are still to be done to use this as potential treatment against UTIs.

Keywords: Antibiotic-resistant bacteria, lytic phages, uropathogenic bacteria.

I. Introduction to UTIs

The term Urinary Tract Infection (UTI) refers to the infection of urinary tract that includes renal pelvis, ureters, bladder and urethra. In case of recurrent UTIs, some associated structures like perinephric fascia, epididymis and prostate may also be infected (Jepsen, 1987). UTI being global health problem effecting 150 million people per year worldwide. It is the 2nd most common bacterial infection after the respiratory tract infections. The infection can be acute, chronic and recurrent abbreviated as aUTIs, cUTIs and rUTIs respectively. These painful
infections reduces the quality of living and results in high social cost for the persons affected (Zalewska-Piątek and Piątek, 2020). Women are more susceptible to UTIs than men due to certain behavioral factors, anatomical factors like short urethra and use of diaphragms and spermicides as birth control pills. Due to low immunity and changing micro-biome, elderly population is also more prone to this infection resulting in high morbidity and mortality (Malik et al., 2020). UTIs are named as cystitis if the infection is in lower urinary tract and pyelonephritis if the infection is in the upper urinary tract. UTIs are clinically categorized as complicated UTIs and uncomplicated UTIs. Uncomplicated UTIs involve the infection in previously healthy individual having normal urinary tract without any structural abnormality or urinary obstruction. Complicated UTIs are the infections in pregnant women, immune compromised people or persons with urinary obstruction or renal failure (Malik et al., 2020).

**Uropathogens- The Causative Agents of UTIs**

Uropathogenic *Escherichia coli* (UPEC) is the most dominant bacterium that is responsible for this infection most of the times, including both complicated UTIs (50-65%) and uncomplicated UTIs (75-85%). Other Gram-positive and Gram-negative bacteria are also the cause of UTIs that includes *Staphylococcus aureus, Staphylococcus saprophyticus, Group B Streptococcus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis* and the yeast, *Candida* species (Malik et al., 2020; Zalewska-Piątek and Piątek, 2020).

Pathogens that are responsible for UTIs have some special features that allow them to grow in urinary tract. This includes the production of adhesins which help them to attach to renal epithelium and bladder, and the production of siderophores, proteases and some toxins which help them to get nutrients from the host. Their biofilm producing ability provides them more resistance against antibiotics due to low metabolic activity in deeper layers of biofilms. These biofilm are able to colonize biotic as well as abiotic surfaces like catheters making catheterized patients more susceptible to infection (Malik et al., 2020).

**Use of Antibiotics and Emergence of Antibiotic-Resistant Strains**

Antibiotic therapy is considered effective for managing UTIs but in recent years, it has become strenuous because of continually increasing resistance of uropathogens especially
UPECs against many antibiotics. Repeated antibiotic treatment is required in chronic or recurrent infections. Also, new and recurrent episodes of UTIs can’t be distinguished by therapeutic regimens. So, antibiotics are being excessively used. Unnecessary and widespread usage of antibiotics even without characterizing the bacteria during the treatment results in emergence of extensively drug resistant (XDR) and multi drug resistant (MDR) strains. *S. aureus* is listed as high priority pathogen by WHO for research of new antibiotics and development of new treatments while UPEC, *K. pneumonia*, *P. aeruginosa* and *P. mirabilis* are listed as priority 1 or critical pathogens against which new treatments are urgently needed (Zalewska-Piątek and Piątek, 2020).

II. Phage Therapy as an Alternative Treatment

Bacteriophages (bacteria eaters) are the viruses that use bacteria as their host for their replication. By taking advantage of their bacteria eating mechanism, they can effectively be used as a treatment strategy against pathogenic bacteria. The practice of using bacteriophages for therapeutic purpose was being developed almost a decade ago, even before the discovery of penicillin. After the discovery and effective use of antibiotics, the use of phages for therapeutic purpose was not the matter of interest anymore. Now, in this so called post-antibiotic era, the idea of phage therapy again gained attraction of many scientists (Lin et al., 2017).

Mode of Action of Bacteriophages

Bacteriophages can infect bacteria in two ways- the lytic cycle and the lysogenic cycle. Infection starts by attachment of phage to specific receptors on the bacterial cell wall. After attachment, they inject their genetic material into the bacterium. In lytic cycle, virus’s genetic material takes up the control of host machinery to replicate itself and promote lysis of bacteria resulting in release of many virus particles. In lysogenic cycle, viral genetic material is inserted to the bacterial chromosomal DNA and replicates along with it resulting in the formation of temperate phage. For therapeutic purpose, lytic phages are preferred due to two reasons. Firstly, they destroy their host by lysis while host is not immediately affected by temperate phage. Secondly, temperate phages can transfer resistance or virulence genes due to integration of their genome into bacterial genome during their life cycle (Parisien et al., 2008).
Emergence of Resistance against Phages

Bacteria can develop resistance against phages by evolving their certain protective mechanisms. Firstly, they can prevent phage entry by hiding, changing or even losing specific phage receptors. Secondly, they can prevent phage attachment by secreting extracellular polymeric substance (EPS), alginates or glyco-conjugates. Thirdly, some natural defense mechanisms like Super infection exclusion (Sie) system, Clustered Randomly Interspaced Short Palindromic Repeats (CRISPR) system or Abortive (Abi) system can interrupt DNA injection, remove viral genetic material or prevent transcription or translation of viral DNA respectively. The phage resistance can be overcome by pre-adapting the phage for several generations according to its host in vitro. In addition, combination of different phages (phage cocktails) or phages in combination with antibiotics can be used which will be described later (Parisien et al., 2008).

III. Ways to Carry Out Phage Therapy against UTIs

Phages can be used in four different ways as anti-microbial agents for treatment of UTIs. These include natural phage cocktails, genetically modified phages, phage lytic proteins and phages in combination with antibiotics (Malik et al., 2020).

Polyphage Therapy or Phage Cocktails

Polyphage therapy is the use of two or more phages instead of a single phage as in monophage therapy. This provides two major benefits- one is the broader host range and second is the delayed resistance. This approach also allows targeting biofilms in the urinary tract. This treatment is effective even after one of the bacterial receptors evolves by mutation. The first phage cocktail against MDR UPECs was composed of two phages isolated from wastewater namely KEP10 phage and T4 phage. So, these were the first effective therapeutic candidates against UTIs (Zalewska-Piątek and Piątek, 2020).

Catheter-associated urinary tract infections (CAUTIs) by P. mirabilis are quite hard to treat due to their highly antibiotic resistant crystalline biofilms over the surface of catheters. Two phages, the myovirus vB_PmiM_5461 and the podovirus vB_PmiP_5460 have shown a
remarkable decrease of biofilm formation even up to 168 hours of catheterization. These results mark the potential of phage cocktails against UTIs (Melo et al., 2016).

Genetically Modified Phages

A new way to treat UTIs is through the use of genetically modified phages having desirable qualities. Phages can easily be engineered by using different genetic engineering methods- CRISPR genome editing system, rebuilding phage genomes in vitro, phage recombinant of electroporated DNA, homologous recombination, in vivo recombinant, assembly from synthetic oligonucleotide or whole-genome synthesis. These genetically modified bacteriophages have been effectively used against UPECs, P. aeruginosa, and S. aureus. Phages have also been genetically engineered to produce the EPS-degrading enzyme for efficient clearing of biofilms. These modified bacteriophages can be used to detect and control bacterial infections in humans being harmless as they are modified in vitro and safe to use (Zalewska-Piątek and Piątek, 2020).

A notable example of this therapy is its use in killing intra-cellular bacteria in human urothelial cells. The fluorescent phage K1F-GFP was created by inserting a green fluorescent gene into the genome of phage K1F infecting E. coli K1 responsible for UTIs using the CRISPR-Cas system. The above phage and E. coli EV36-RFP (a K-12/K1 hybrid displaying the K1 capsule) can invade epithelial cells of the urinary bladder by phagocytosis. Inside the cells, bacteria were killed by engineered phage K1-GFP. Moreover, the bacteria and viruses were degraded by LC3-associated phagocytosis and autophagy (Møller-Olsen et al., 2018).

Phage Lytic Proteins

Phage lytic enzymes (PLEs) are quite fascinating to be used as antibacterial molecules against UTIs. They are of two kinds- Virion-associated lysins (VALs) and endolysins. VALs are the enzymes attached to the virus that helps in the degradation of bacterial cell surface from outside, thus allowing the phage genetic material to be injected. Endolysins are the enzymes that degrade the bacterial cell by attacking the peptidoglycan (PG) from within, thus allowing the viral progeny to be released from the cell (Malik et al., 2020). Earlier, the use of PLEs was limited to gram positive bacteria due to their inability to cross the outer membrane that is present around gram negative bacteria. It is important to note that most of the uropathogens are gram
negative. So, its use was narrow against UTIs. Recently, researchers report the effectiveness of using outer membrane disrupting peptides or chelating agents like ethylene diamine tetraacetic acid (EDTA), disodium salt dihydrate in combination with PLEs to overcome outer membrane barrier in gram negative bacteria. PLEs can also be genetically engineered to improve their ability to reach PG layer of bacterial cell for enhanced anti-bacterial activity against gram negative bacteria.

A remarkable example of this therapy is the use of endolysin LysPA26 in combination with EDTA that gives good antibacterial activity against clinically isolated MDR strains of UPEC, *P. aeruginosa* and *K. pneumonia* (Malik *et al.*, 2020).

**Phages in Combination with Antibiotics**

Therapies that involve the use of phages in combination with low dose of antibiotics show a synergistic response and are more effective against pathogens as compared to phage preparations alone. This mechanism is termed as phage-antibiotic synergy (PAS). It results in increased number of virulent phages and increased size of phage plaques (Zalewska-Piątek and Piątek, 2020). Kim et al first explained the underlying phenomenon by delayed lysis of bacteria. They concluded that sub-lethal concentration of antibiotics leads to elongation of bacteria which subsequently decreases the holin (the protein responsible for the formation of pores in bacterial cytoplasmic membrane) concentration near bacterial membranes. This low conc. delays the process of lysis which ultimately results in increased number of phage particles within the cell (Kim *et al.*, 2018).

The principal study on synergism was reported in 2007 against UPECs. Later on, many studies were done against other biofilm forming uropathogens like *P. aeruginosa* and *K. pneumoniae*. All of them showed remarkable reduction in cell density. Till now, these studies are restricted to some human cell lines and a few animal models. So, more successful trials are still needed to use it as acceptable therapy against UTIs (Malik *et al.*, 2020).

**IV. Routes of Administration for Phage Therapy**

Phages can be administrated in many different ways. There is no specific guideline about how to deliver phages for best clinical results. Different studies used varied routes of
administration depending on the type of infection and the phage being used for the treatment. A lot of work is still to be done in this regimen (Chegini et al., 2021). The benefits and drawbacks of different delivery routes are summarized in the Table 1. According to a study, the most effective routes of administration in case of UTIs are oral and intrarectal which resulted in effective microbiological response in 50% of the cases (González-Villalobos et al., 2022).

**Table 1: Different delivery routes for phage therapy**

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Benefits</th>
<th>Drawbacks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>Ease of delivery</td>
<td>Phage titer can be reduced by stomach acids.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Greater dosage volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerosol</td>
<td>Ease of delivery</td>
<td>Chances of phages to be lost in higher proportion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Effective when need to deliver to poorly perfused areas of lungs</td>
<td>Phages can be entrapped by mucus</td>
<td></td>
</tr>
<tr>
<td>Topical</td>
<td>High amount of phage directly at the infection site</td>
<td>Can escape from the target site if suspended in liquid</td>
<td></td>
</tr>
<tr>
<td>Suppository (Intrarectal)</td>
<td>Slow release of phages</td>
<td>Chances of insufficient dose</td>
<td></td>
</tr>
<tr>
<td>Intravenous</td>
<td>Rapid diffusion</td>
<td>Risk of clearance by immune system</td>
<td>Romero-Calle et al., 2019</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>Delivery to the infection site</td>
<td>Less dosage volume</td>
<td></td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>Diffusion to multiple sites</td>
<td>Inaccurate estimate of extent of diffusion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Greater dosage volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>Localized diffusion</td>
<td>Less dosage volume</td>
<td></td>
</tr>
</tbody>
</table>

V. Clinical Use of Phage Therapy Against UTIs

Bacteriophages are being clinically used since twentieth century in some countries like Russia and Georgia but poorly documented in literature. In the present era, extensive research on this topic is also being done in western countries and all other regions of the world to overcome the problem of antibiotic resistance (González-Villalobos et al., 2022).

**Studies in Vitro**

In a study, *E. coli* and *K. pneumonia* strains were isolated from patients infected with UTIs. These strains were then tested in vitro to check their susceptibility against commercially...
available bacteriophage cocktails using spot-test method. As a result, the lytic activity was found to be varied between 66% and 93% which is quite promising (Sybesma et al., 2016). Another study on UPECs demonstrated that only a single dose of three-phage cocktail is enough for its eradication up to a greater extent (Bolocan et al., 2016).

Animal Model

Nishikawa et al. (2008) applied phage therapy to a mouse model. The lethal dose of UPEC was introduced into the bladder. The authors note that phages were released in urine when injected into the peritoneal cavity. The effectiveness of phage in disease control varies depending on the type of phage and the dose. The best results were achieved with phage T4 that lead to 100% survival of mouse. Using the same method of administration, two phages were proved to be effective against UTIs caused by Cronobacter turicensis. This cocktail significantly reduced the mouse load by 70%. In addition, symptomatic relief was also observed using phage treatment (Melo et al., 2020).

Clinical Trials on Human Beings

There are very few controlled clinical trials of phage therapy on human patients as compared to studies in vitro or in animal models (Kortright et al., 2019). Some of them are summarized in the Table 2.

VI. Phage Therapy Vs. Antibiotic Therapy

Phages and antibiotics both function as antibacterials as they lead to disruption or death of bacterial colonies either through inhibition or lysis of bacteria. The prime differences between them makes each therapy less or more suitable according to a certain situation. Some of the benefits and drawbacks of phage therapy over antibiotics are discussed below (Lin et al., 2017).

Advantages of Phage Therapy

Phages have several advantages over antibiotics which makes them an efficient tool for therapeutic purpose. Some of them are described as follows. Phages can replicate inside the host due to which their low dosage is enough while many molecules of antibiotics are required. Polysaccharide depolymerizes on tail structures of some phages help to infect bacteria in hard to
reach areas making them effective against bacterial biofilms while antibiotics cannot hydrolyze extracellular polymeric substance and are ineffective against biofilms. Phages are highly specific in terms of host range and regarded as safe as they cannot infect eukaryotic cells or normal flora while antibiotics have many side effects as they disturb normal flora of human body (González-Villalobos et al., 2022). The inherent toxicity of phages is also very low as they comprised only of proteins and nucleic acid (Loc-Carrillo and Abedon, 2011). Phages being natural can evolve as the bacteria evolve overcoming the problem of resistance in bacteria while antibiotics being static and artificial cannot evolve. Mutation frequency of phage genome is

Table 2: Different clinical trials of phage therapy against UTIs

<table>
<thead>
<tr>
<th>Target bacteria</th>
<th>Phage dose</th>
<th>Delivery route</th>
<th>Treatment duration</th>
<th>Outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>2*10^7 PFU after every 12h</td>
<td>Infused directly into the bladder</td>
<td>10 days</td>
<td>Condition improved, 10-fold decrease in bacterial load after 5 days and sterile urine samples after complete treatment.</td>
<td>(Khawaldeh et al., 2011)</td>
</tr>
<tr>
<td><em>Staphylococcus E. coli P. mirabilis</em></td>
<td>-</td>
<td>Bladder irrigation Intravesically</td>
<td>-</td>
<td>No superiority over placebo or antibiotic therapy</td>
<td>(Górski et al., 2020)</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>10ml of phage preparation twice a day</td>
<td>Intrarectal</td>
<td>28-33 days</td>
<td>Symptomatic relief along with successful eradication of pathogen</td>
<td>(González-Villalobos et al., 2022)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>Phage cocktail</td>
<td>Bladder irrigation</td>
<td>-</td>
<td>Phage resistant bacteria were found</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phages + Antibiotics</td>
<td>Bladder irrigation with phage preparation and oral antibiotics</td>
<td>-</td>
<td>Symptoms disappeared</td>
<td>(Chegini et al., 2021)</td>
</tr>
</tbody>
</table>
comparatively greater than bacterial genome which makes phage therapy more reliable than antibiotic therapy as phage selection and isolation is also cheaper and shorter than discovering new antibiotics. Phages also offers economic benefits as their production cost is relatively less than that of antibiotics (Domingo-Calap and Delgado-Martínez, 2018).

**Concerns Regarding Phage Therapy and Their Possible Solutions**

Some potential drawbacks of phage therapy over antibiotics are described as follows. Being very specific, phages have a very narrow host range. The infection causing bacterium must be identified prior to treatment because specific phage will be required against each bacterial infection. This issue can be resolved by using phage cocktails which widens the host range (Domingo-Calap and Delgado-Martínez, 2018). Phages can be neutralized by immune system of host which results in failure of treatment but usually single dose is not enough to generate an immune response while regarded as highly effective against infectious bacteria. In few cases, if more than one dose is required to eliminate bacteria, long circulating phages can be used that have the ability to avoid immune response in host (Parisien et al., 2008).

Rapid lysis of bacterial cells may lead to the release of excessive amount of bacterial endotoxins but Hangens et al showed that recombinant phages can be constructed to minimize the release of endotoxin (Hagens and Bläsi, 2003). Bacteria that contain the genome of lysogenic phage as a prophage may result in acquired resistance against the corresponding lytic phage which is termed as lysogenic immunity. In this way, bacterial virulence may also be altered. Moreover, phages may transfer harmful genes from one bacterium to other. During excision of prophage genome, some portion of bacterial genome can also be taken up which may code for toxins. To overcome these issues, choice of phage must be very appropriate (Parisien et al., 2008). Another obstacle in the way of phage therapy is the lack of pharmacokinetic (action of body on the phages) and pharmacodynamics (action of phages in the body) data. Also, there is need to have much more information about the route of administration or doses in case of each specific phage (Domingo-Calap and Delgado-Martínez, 2018).
Conclusion

Phage therapy has many benefits over antibiotics but there is a lot of research gap regarding phage selection, dose and treatment duration. Controlled clinical trials are needed prior to use this as potential treatment for UTIs in the fight against antibiotic resistance.

References


