A Novel Phage Cocktail Therapy of Urinary Tract Infection in Mouse Model

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Abstract

Escherichia coli (E. coli) is the major bacterial pathogens associated with many cases of serious infections such as urinary tract infections (UTI), meningitis intestinal, and etc. The rapid emergence of antimicrobial multidrug-resistant bacteria occurring worldwide has been attributed to the overuse of antibiotics. Alternative strategies must be developed to overcome antibiotic resistance. A promising alternative for the treatment of infections is the use of phages as antibacterial agents. 90 female albino mice randomly divided into three groups (n=30) and were used for the induction of UTI. The animals were acclimatized in their cages for 24 h before inoculation and allowed to access chow and water freely. For UTI induction the periurethral area was sterilized with 70% ethanol then bacterial inoculation was injected into the bladder through urethra by using a sterile Teflon catheter of 24 gauge with an outer diameter of 0.7 mm and length of 19 mm. A single phage and a phage cocktail preparation have been evaluated for their therapeutic activity in the mouse model of chronic urinary tract infection UTI induced by transurethral injection of two isolates of the uropathogenic E. coli 8 and E. coli 302. The results of the transurethral and intra-peritoneal injection of phage(s) that prepared on day 10 after the establishment of the mouse chronic model, showed no effect of a single phage PEC80 in the treatment of UTI, whereas both administration routes of the phage cocktail preparation resulted in clearance of bacteria from mice urine and homogenates of the urinary bladders and kidneys of the sacrificed mice after a period of 24 h of administration of phage cocktail dose. The high activity of the phage cocktail in the treatment of mouse chronic model of UTI is attributed to the broader host range of the phage cocktail compared with the very narrow host range of the phage PEC80. It is concluded that phage therapy by using phage
preparations as the 25 phages cocktail evaluated in this study is a highly promising and potential alternative therapy for human UTIs.

**Keywords:** Phage Therapy, Uti, Drug Resistance, Phage Cocktail, Alternative Therapy

1. **Introduction**

Escherichia coli (*E. coli*) is the bacterial pathogens linked with many cases of infections disease. *E. coli* is a non-pathogenic commensal bacterium categorized by its versatility and assortment once it is capable of colonizing human and other animal gastrointestinal systems. However, new virulent strains appear due to the evolution of some strains, which are responsible for varied diseases, such as urinary tract infections (UTI), pneumonia, and etc.. UTI are one of the most common infections affecting humanity, especially women. The rapid emergence of antimicrobial multidrug-resistant bacteria occurring worldwide has been attributed to the overuse of antibiotics. Currently, the increased occurrence and prevalence of antibiotic resistance in *E. coli* is a particular concern. One of the most problematic areas of drug resistance is the resistance acquired by fluoroquinolones and third generation cephalosporin by Enterobacteriaceae, which include strains of *E. coli*, according to the World Health Organization (WHO). For the reduction of the development and dissemination of microbial resistance, alternative strategies must be developed. A promising alternative for the treatment of infections is the use of phages as antibacterial agents, mainly those caused by multidrug-resistant bacteria\(^\text{(1)}\).

Bacteriophage therapy is one of the most outstanding alternatives for antibiotics in the treatment of all bacterial infections. Phages were first described to be active in the treatment of bacterial pathogens in 1917 by Felix d’Herelle, since that time, phages were studied extensively for their bioactivity application in the treatment of different human infections\(^\text{(2)}\). However, attention on the therapeutic potential of phages subsided dramatically after the wide use of antibiotics in the 1940s, but many phages worker in the former Soviet Union continued paying their attention to the application of phages alone in the treatment of human bacterial pathogens\(^\text{(2)}\).

Nowadays, bacteriophage therapy took great attention from the global scientific society and scientific research and became one of the most fast-developed areas of scientific research and interest owing to the urging demand to dissolve common problems of drug resistance of bacterial pathogens towards various human infections, including UTIs\(^\text{(3)}\).
The objectives of this study are to establish a mouse model of chronic UTI, a preparation of monovalent and polyvalent phage preparations active against uropathogenic E. coli isolates, treatment of the mouse model of chronic UTI by transurethral and intraperitoneal administration of monovalent and polyvalent phage preparations and, evaluation of results.

2. Materials and Methods

2.1. Bacteria and phages

A number of 25 isolates of UroPathogenic Escherichia coli (UPEC) and a number of 25 phages lytic against the UPEC isolates have been isolated and characterized in a previous study conducted by (4). The phages are PEC3, PEC11, PEC15, PEC16, PEC28, PEC30, PEC36, PEC37, PEC38, PEC44, PEC51, PEC52, PEC55, PEC63, PEC68, PEC78, PEC80, PEC94, PEC102, PEC133, PEC215, PEC301, PEC304, PEC305 and PEC306. Every single phage showed lytic activity against E. coli isolates in a percentage of 27% or less, whereas the phage cocktail composed of the 25 phages showed 100% activity against all E. coli isolates(4).

The bacteria were isolated from human UTIs cases. The urine samples were cultured, purified, and diagnosed with slide morphology, growth on differential media, IMViC, and Vitik2 automated system. The presence of P fimbria and type 1 fimbriae was detected by mannose resistance blood agglutination (human type O blood), mannose sensitive blood agglutination (Guinea pig blood), respectively(5). The capsule formation was detected by capsule stain and the hemolysin production by blood agar plate (5%) (6).

2.2. Phage cocktail preparation

A monovalent phage and polyvalent phage preparations were used to treat the mouse model of chronic UTI induced by the UPEC. A monovalent phage preparation of phage PEC80 was prepared in a concentration of $10^7$ PFU/ml in the SM buffer (Sigma-Aldrich, USA). A number of 25 phages, which showed high activity against uropathogenic E. coli isolates, were mixed to prepare a polyvalent phage cocktail. A concentration of $10^7$ PFU/ml in the SM buffer for every of the 25 bacteriophage preparations were mixed in equal volumes to give a total concentration of a phage cocktail of $10^7$ PFU/ml.

2.3. Inoculums preparation
The UPEC was cultured in membrane filter-sterilized human urine to strengthen their adaptation to human urine\(^7\). The bacterial culture was incubated at 37°C for 18 hrs with shaking (200 rounds/min). The bacterial suspension was centrifuged (at 7000 rpm/min for 10 min.) and the bacterial pellet was resuspended in phosphate buffer saline to approximately \(10^{10}\) CFU/ml.

**2.4. Animals**

90 female albino mice randomly divided into three groups (n=30) and were used for the induction of UTI. The animals were acclimatized in their cages for 24 h before inoculation and allowed to access chow and water freely.

**2.5. Ethical treatment with animals**

An animal model study was done under recommendations of ethics of animal model commission of Iraqi ministry of higher education and scientific research.

**2.6. Establishment of mouse chronic UTI**

The sodium pentobarbital was used to anesthetize the mice in a concentration of 0.05 mg/ml prior to the transurethral injection of the bacterial suspension. The periurethral area was sterilized with 70% ethanol. Bacterial inoculation was injected into the bladder through urethra by using a sterile Teflon catheter of 24 gauge with an outer diameter of 0.7 mm and length of 19 mm (Sigma-Aldrich). In order to establish chronic UTI in inoculated mice, the bladder mucosa of mice was traumatized before inoculation by injecting the urinary tract with 100 µl of HCl solution (0.1 N), for 45 seconds. Then, the acidic urinary tract was neutralized by injection of 100 µl of KOH (0.1 N) and flushed with sterile saline by a tuberculin syringe [8]. After traumatization, a bacterial inoculation of \(1 \times 10^6\) to \(2 \times 10^6\) CFU (20 µl) was injected into the urinary bladder through a Teflon catheter for 30 seconds via a microsyringe (Sigma-Aldrich) 24 h after the bladder mucosa traumatization. Two models of a mouse chronic UTI were induced, a chronic UTI by a single strain of *E. coli*, which was induced by the UPEC isolate *E. coli* 80 and a multiple strains model of a mouse chronic UTI induced by two isolate of *E. coli*, which induced by an inoculum of combined \(1 \times 10^6\) organisms for each isolate of *E. coli* 8 and *E. coli* 302. The urine samples were collected from 3 infected, randomly selected mice on intervals of 2 days starting from day 1 after mouse infection up to day 30 and cultured for detection UPEC isolate(s).
The same 3 selected mice on each interval were sacrificed. The kidneys and urinary bladders homogenates of sacrificed mice were cultured, diagnosed, and the number of bacteria for each organ was calculated\(^{(8, 9)}\).

### 2.7. Treatment of mouse chronic UTI induced by a single strain of uropathogenic \textit{E. coli}

A mouse model of chronic UTI induced by a single strain of UPEC was treated with a single dose of 100 µl of monovalent phage preparation of phage PEC80 administered transurethral on a group (30 mice), and intraperitoneally on another group (30 mice) on day 10 after infection. The urine samples were collected from 3 mice daily from day 10 up to day 20 after infection. Same selected 3 mice on each day were sacrificed. Their bladders and kidneys were homogenate, cultured, diagnosed for uropathogenic \textit{E. coli} and the number of bacteria for each organ was calculated.

### 2.8. Treatment of mouse chronic UTI of multiple strains of uropathogenic \textit{E. coli}

A mouse model of chronic UTI induced by multiple strains of uropathogenic \textit{E. coli} was treated by a single dose of a volume of 100 µl of polyvalent phage preparation administered transurethral on a group of 30 mice, and intraperitoneally on another group of 30 mice on day 10 after infection. Another group of 30 mice was injected transurethral and peritoneally with monovalent phage preparation of phage PEC80 for comparison. The urine samples were collected from 3 mice daily from day 10 up to day 20 after infection. The same selected 3 mice on each day were sacrificed. Their urinary bladders and kidneys were homogenate, cultured, diagnosed for the presence of uropathogenic \textit{E. coli} and, the number of bacteria for each organ was calculated.

### 2.9. Bacterial tests

The daily collected urine and homogenates of urinary bladders and kidneys of sacrificed are cultured for detection on UPEC. The isolated bacteria were detected for the presence of K and O antigens, P fimbriae, type 1 fimbria, and the production of hemolysin. The K and O antigens are detected by agglutination with goat polyclonal to K+ O antigens (Abcam, England)\(^{(10, 11)}\). At each interval specified for calculation of viable bacteria urinary bladders and kidney of treatment under evaluation, a number of 3 mice are sacrificed and their bladders and kidneys
were placed in a grinding tube containing 1 and 5 ml of sterile normal saline for urinary bladders and kidneys, respectively, and homogenized with Teflon grinder. The colony-forming units of UPEC per organ was calculated through serial dilution of homogenate and plating of a volume of 50 µl from each dilution was plated on DHL agar (Sigma-Aldrich, USA) to select the UPEC. The number of CFU/organ was calculated as the mean number of bacteria for each organ ± the standard deviation (SD)\(^9\).  

3. Results and Discussion

3.1. Establishment of mouse chronic UTI

The daily urine culture showed the positive culture of \textit{E. coli} in mice urine beginning from 1 day after infection. Culture results of bladders and kidneys homogenates of sacrificed mice were at intervals of days (1, 3, 5, 7, 10, 14, 24 and 30) after infection showed slight variation in the number of bacteria. Figures 1 and 2 show the mean of culture results of urinary bladders, and kidneys of 3 mice at each interval during the period of mouse chronic UTI. The minimum number of bacteria detected by this procedure was 100 CFU for the kidney and 20 CFU for the bladder.

![Figure 1](image)

Figure 1. The culture results of bladder homogenates of mice sacrificed on days (1, 3, 5, 10, 15, 20, 25, and 30) of infection establishment for both, mice with traumatized bladder mucosa (♦), and non-traumatized bladder mucosa (■).
Figure 2. Culture results of kidney homogenates of mice sacrificed on days (1, 3, 5, 10, 15, 20, 25, and 30) of infection establishment for both, mice with traumatized bladder mucosa (●), and non-traumatized bladder mucosa (■).

3.2. The treatment of the chronic mouse UTI induced by a single strain of uropathogenic *E. coli*

The treatment of a mouse chronic UTI induced by a single isolate of *E. coli* 80 by a single dose of phage preparation of PEC80 resulted in clearance of bacteria from urine culture and cultures of urinary bladders and kidneys homogenates after 24 hours only (Figures 3 and 4).

Figure 3. Culture results of bladder and kidney homogenates of mice sacrificed on days (10, 11, 12, 13, 14, and 15) of infection establishment after transurethral administration of single phage preparation of phage PEC80 on day 10.
3.3. The treatment of the chronic mouse UTI induced by multiple strains of uropathogenic *E. coli*

The treatment of mouse chronic UTI induced by *E. coli* 8 and *E. coli* 302 by a single dose of phage cocktail preparation resulted in clearance of bacteria from urine culture and culture of urinary bladders and kidneys homogenates after 24 hr. only, whereas injection of mice transurethral and peritoneally with single phage preparation of phage PEC80 had no effect on urine culture results in no results of culture of urinary bladders and kidneys homogenates (Figures 5, 6, 7 and 8).

Figure 5. The culture results of bladder homogenates of mice sacrificed on days (10, 11, 12, 13, 14, and 15) of infection establishment after transurethral administration of phage...
preparation on day 10. Mice injected with phage cocktail preparation (♦), Mice injected with single phage preparation (■)

Figure 6. The culture results of kidney homogenates of mice sacrificed on days (10, 11, 12, 13, 14, and 15) of infection establishment after intraperitoneal administration of phage preparation on day 10. Mice injected with phage cocktail preparation (♦), Mice injected with single phage preparation (■)

Figure 7. The culture results of bladder homogenates of mice sacrificed on days (10, 11, 12, 13, 14, and 15) of infection establishment after intraperitoneal administration of phage preparation on day 10. Mice injected with phage cocktail preparation (♦), Mice injected with single phage preparation (■)
Figure 8. The culture results of kidney homogenates of mice sacrificed on days (10, 11, 12, 13, 14, and 15) of infection establishment after intraperitoneal administration of phage preparation on day 10. Mice injected with phage cocktail preparation (♦), Mice injected with single phage preparation (■)

The chronic model of mouse UTI is the experimental model of UTI that is characterized by the presence of a concentration of $10^6$ CFU of bacterial pathogen in the urinary tract (urinary bladder and kidney) that lasts for more than 3 weeks after the challenge. This chronic model is successfully induced in mice via traumatization of their urinary tract with HCL for 45 seconds followed by neutralization with KOH. The activity of phage preparations against a mouse model of uropathogenic *E. coli* was detected on traumatized bladder mucosa model because the non-traumatized bladder mucosa model resulted in a transient infection that transformed normally into mild within just 7 days after the challenge, and could not depend on onevaluation the activity of the phage preparations, whereas, the traumatized bladder mucosa model resulted in chronic infection that lasts for more than 30 days with just little drop in the number of bacteria recovered from a urine culture, or homogenates of bladder and kidney. No systemic infection was developed through the course of mouse UTI.

The success of treatment of mouse experimental model by intraperitoneal and transurethral administration of both single, and phage cocktail preparations are indicated for the potential usage of those phages in treatment of human UTI cases by parenteral injection, besides other routes of phage administration, and this property makes such treatment simple and easy for routine work just like chemotherapy.

The success of phage PEC80 in treatment of mouse model of UTI induced by the host bacterium *E. coli* 80 and failure of the same phage in treatment of the model induced by *E. coli*
8 and *E. coli* 302 was attributed to the narrow host range of this phage that limits the advantage of such phages in the phage therapy of bacterial infection, but the success of the 25 phages cocktail in treatment of mouse model of both strains was due to its broad-host-range against all *E. coli* isolates that obtained from combination of various mechanisms of phage adherence and lysisto target bacterium\(^{(16-19)}\).

The multi-drug resistance and extensive drug resistance properties of uropathogenic *E. coli* exaggerate the importance of the success of phage preparations in the eradication of such pathogens. The failure of antibiotics in the treatment of such infections leads to their dissemination to other sites of the body and becoming life-threatening\(^{(15)}\).

The importance of such preparation is not concerned only with antibiotic resistant pathogens, but also with the cases of UTIs in women during their pregnancy and perinatal period, in which antibiotic- administration to treat UTIs will be so risky for embryo, fetus and newborn babies\(^{(20)}\).

The activity of the 25 phages cocktail on most *E. coli* isolates makes it a strong candidate against all *E. coli* infections of other pathotypes of *E. coli* like enteropathogenic *E. coli*, Enterotoxigenic *E. coli*, and Enter invasive *E. coli*, which represents life-threatening conditions, especially, in the cases of multi-drug and pan-drug resistant strains\(^{(21)}\).

In Conclusion,

- Phagecocktail is the optimal way among other therapies to expand the host-range of phages active against UPEC and other bacterial pathogens of UTIs. Such expansion of the hostrange made it possible to employ phage therapy as a potent, promising, and alternative therapy for cases of UTIs.
- The success of the treatment of the mouse model of chronic UTI by phage cocktail is showing strong indication for promising possibility of phage cocktail in treatment of UTIs via transurethral and intra-peritoneal route.
- The simplicity and rapidity of phage therapy in the treatment of chronic UTI was remarkable property for this method of therapy and could be even superior over classical antibiotics in the case of application of phage therapy with proper standards.

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Conflict of interest

None

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