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Original Article

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

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Abstract

Escherichia coli (*E. coli*) is a major bacterial pathogen associated with many cases of serious infections, such as urinary tract infections (UTI) and meningitis intestinal. 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. A total of 90 female albino mice were randomly divided into three groups (n=30) and 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 peri-urethral area was sterilized with 70% ethanol, and bacterial inoculation was then injected into the bladder through the urethra using a 24-gauge sterile Teflon catheter 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 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 the clearance of bacteria from mice urine and homogenates of the urinary bladders and kidneys of the sacrificed mice after 24 h following the 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 to the very narrow host range of the phage PEC80. It is concluded that the 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 a bacterial pathogen linked with many cases of infectious diseases. *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), and pneumonia. The UTI is 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 the trains of *E. coli* according to the World Health Organization.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 (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 (2). However, attention on the therapeutic potential of phages subsided dramatically after the wide use of antibiotics in the1940s; however, 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 (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 surging demand to dissolve common problems of drug resistance of bacterial pathogens towards various human infections, including UTIs (3).

The objectives of this study are the establishment of a mouse model of chronic UTI, preparation of monovalent and polyvalent phage 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 *E. coli* (UPEC) and a number of 25 lytic phages 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 a lytic activity against *E. coli* isolates in a percentage of 27% or less, whereas the phage cocktail composed of 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. Differential media, IMViC, and Vitik2 automated system were used for the growth. The presence of P fimbria and type 1 fimbria was detected by mannose resistance blood agglutination (human type O blood) and 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

The monovalent 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 each of the 25 bacteriophage preparations was 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 h 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 1010 CFU/mL.

2.4. Animals

A total of 90 female albino mice were randomly divided into three groups (n=30) and 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. Establishment of Mouse Chronic UTI

The sodium pentobarbital was utilized to anesthetize the mice in a concentration of 0.05 mg/ml prior to the transurethral injection of the bacterial suspension. The peri-urethral area was sterilized with 70% ethanol. Bacterial inoculation was injected into the bladder through the urethra using a 24-gauge sterile Teflon catheter 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 sec. Subsequently, the acidic urinary tract was neutralized by the 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 1×10^6 to 2×10^6 CFU (20 µl) was injected into the urinary bladder through a Teflon catheter for 30 sec via a micro syringe (Sigma-Aldrich) 24 h after the bladder mucosa traumatization. Two induced models of a mouse chronic UTI included a chronic UTI by a single strain of *E. coli*, which was induced by the UPEC isolate (*E. coli* 80), and a multiple strain model of a mouse chronic UTI induced by two isolates of *E. coli*, which was induced by an inoculum of combined 1×10^6 organisms for each isolate of *E. coli* 8 and *E. coli* 302.

The urine samples were collected from three infected and randomly selected mice at intervals of two days starting from day 1 after mouse infection up to day 30 and cultured for the detection of the UPEC isolate(s). The same three selected mice were sacrificed at each interval. The kidney and urinary bladder homogenates of the sacrificed mice were cultured and diagnosed; moreover, the number of bacteria was calculated for each organ (8, 9).

2.6. Treatment of Mouse Chronic UTI Induced by a Single Strain of Uropathogenic *E. coli*

A mouse model of chronic UTI induced by a single strain of UPEC was treated by a single dose of 100 µl of monovalent phage preparation of phage PEC80that was administered transurethrallyon a group (30 mice), and intraperitoneally on another group (30 mice)on day 10 after infection. The urine samples were collected from three mice daily from day 10 up to day 20 after infection. The same selected three mice were sacrificed on each day. Their bladders and kidneys were homogenized, cultured, and diagnosed for uropathogenic *E. coli*; moreover, the number of bacteria was calculated for each organ.

2.7. Treatment of Mouse Chronic UTI of Multiple Strains of Uropathogenic *E. coli*

A mouse model of chronic UTI induced by multiple strains of uropathogenic *E. coli*was treated by a single dose of a volume of 100 µl of polyvalent phage preparation administered trans-urethrally 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 three mice daily from day 10 up to day 20 after infection. The same selected three mice were sacrificed on each day. Their urinary bladders and kidneys were homogenized, cultured, and diagnosed for the presence of uropathogenic *E. coli*; moreover, the number of bacteria was calculated for each organ.

2.8. Bacterial Tests

The daily collected urine and homogenates of urinary bladders and kidneys of the sacrificed were 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 were detected by agglutination with goat polyclonal to K+O antigens (Abcam, England) (10, 11).At each interval specified for the calculation of viable bacteria urinary bladders and kidney of treatment under evaluation, three mice were 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. Following that, they were homogenized with a Teflon grinder. The colony-forming units of UPEC per organ were calculated through serial dilution of homogenate, and plating of a volume of 50 µL from each dilution was placed 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 the Mouse Chronic UTI

The daily urine culture showed the positive culture of *E. coli* in mice urine beginning from day 1 after infection. Culture results of the bladder and kidney homogenates of the sacrificed mice at the intervals of 1, 3, 5, 7, 10, 14, 24, and 30 days after infection showed a slight variation in the number of bacteria. Figures 1 and 2 show the mean of culture results of urinary bladders and kidneys of three 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.

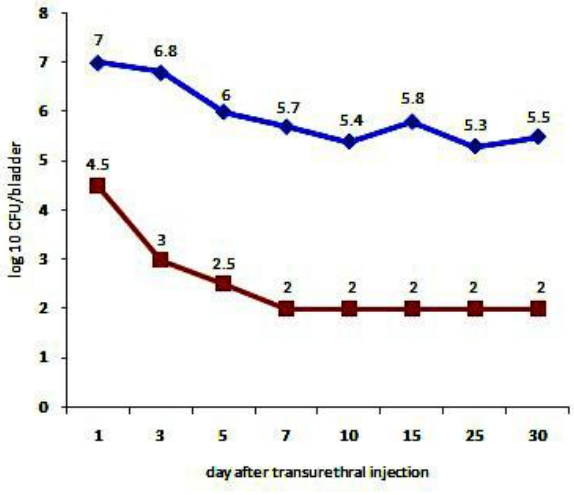

Figure 1. Culture results of the bladder homogenates of mice sacrificed on days 1, 3, 5, 10, 15, 20, 25, and 30 of infection establishment for both mice with traumatized bladder mucosa (♦) and non-traumatized bladder mucosa (■)

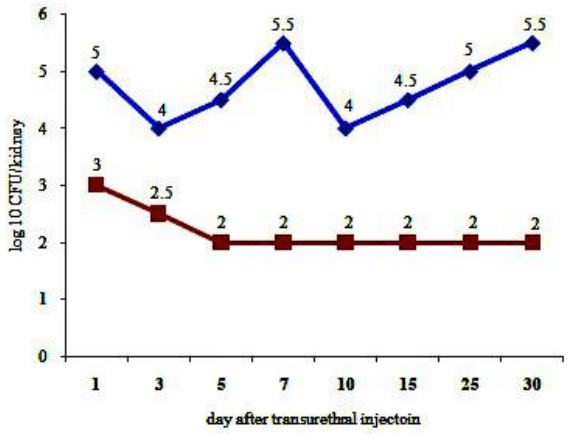

Figure 2. Culture results of kidney homogenates of mice sacrificed on days 1, 3, 5, 10, 15, 20, 25, and 30 of infection establishment for both mice with traumatized bladder mucosa (♦) and non-traumatized bladder mucosa (■)

3.2. 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 and a single dose of phage preparation of PEC80 resulted in the clearance of bacteria from urine culture and cultures of the urinary bladder and kidney homogenates after 24 hours only (Figures 3 and 4).

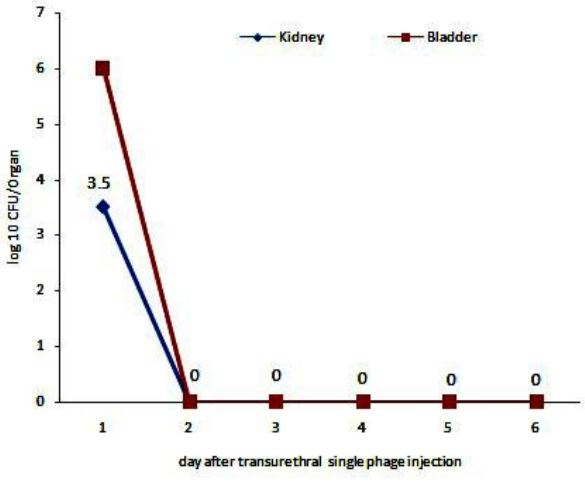

Figure 3. Culture results of bladder and kidney homogenates of mice sacrificed on days 10, 11, 12, 13, 14, and 15of the infection establishment after transurethral administration of single phage preparation of phage PEC80 on day 10

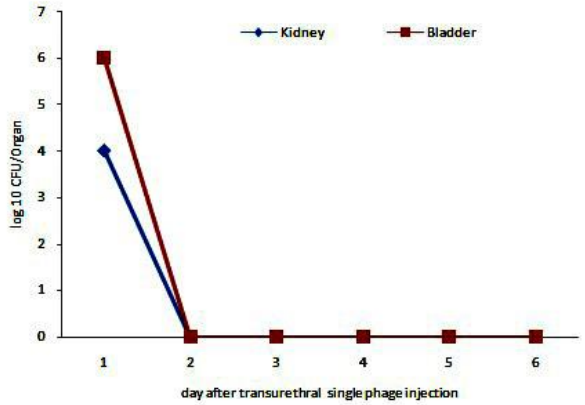

Figure 4. Culture results of bladder and kidney homogenates of mice sacrificed on days 10, 11, 12, 13, 14, and 15 of the infection establishment after intraperitoneal administration of phage cocktail preparation on day 10

3.3. 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 and a single dose of phage cocktail preparation resulted in the clearance of bacteria from urine culture and culture of the urinary bladder and kidney homogenates after 24 h only, whereas the transurethral and peritoneally injection of mice with a single phage preparation of phage PEC80 had no effect on the urine culture and resulted in no culture of the urinary bladder and kidney homogenates (Figures 5, 6, 7, and 8).

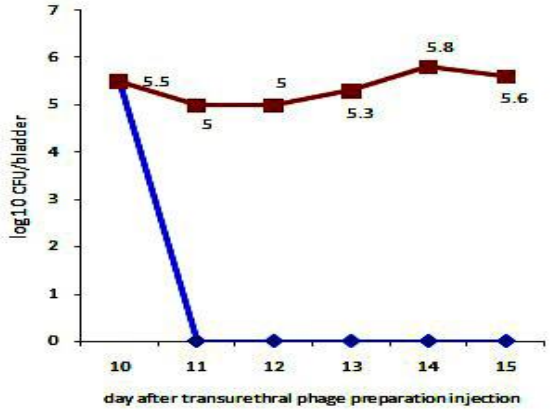

Figure 5. Culture results of bladder homogenates of mice sacrificed on days10, 11, 12, 13, 14, and 15 of the infection establishment after transurethral administration of phage preparation on day 10. Mice injected with phage cocktail preparation (♦), and mice injected with single phage preparation (■)

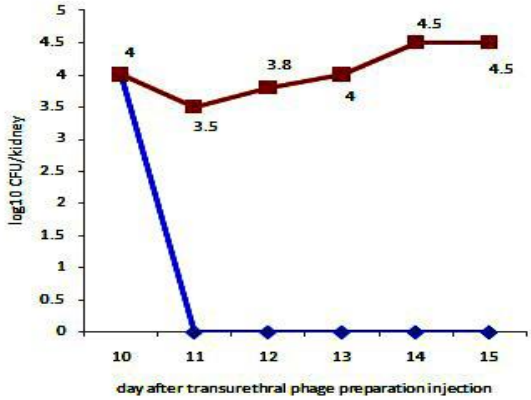

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Figure 6. Culture results of kidney homogenates of mice sacrificed on days 10, 11, 12, 13, 14, and 15 of the infection establishment after intraperitoneal administration of phage preparation on day 10. Mice injected with phage cocktail preparation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

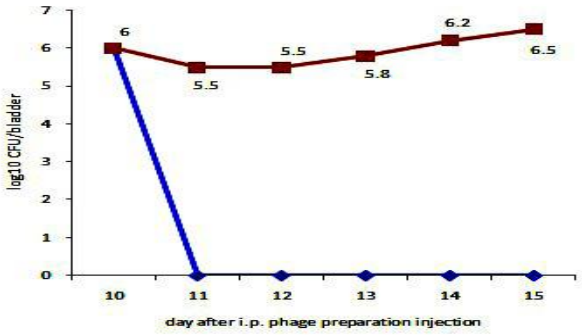

Figure 7. Culture results of bladder homogenates of mice sacrificed on days 10, 11, 12, 13, 14, and 15 of the infection establishment after intraperitoneal administration of phage preparation on day 10. Mice injected with phage cocktail preparation (♦), and mice injected with single phage preparation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

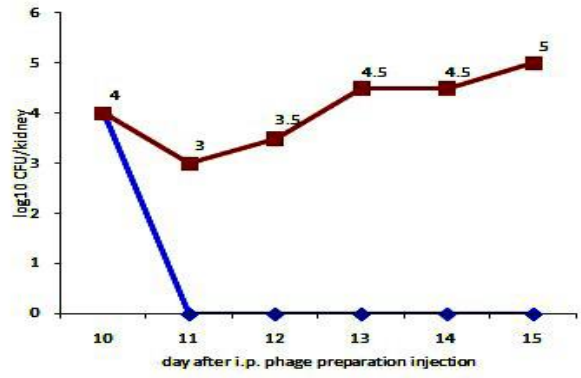

Figure 8. Culture results of kidney homogenates of mice sacrificed on days 10, 11, 12, 13, 14, and 15 of the infection establishment after intraperitoneal administration of phage preparation on day 10. Mice injected with phage cocktail preparation (♦), and mice injected with single phage preparation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

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 three weeks after the challenge (8). This chronic model is successfully induced in mice via the traumatization of their urinary tract with HCL for 45 sec, followed by neutralization with KOH (8).The activity of phage preparations against a mouse model of uropathogenic *E. coli* was detected on traumatized bladder mucosa model since the non-traumatized bladder mucosa model resulted in a transient infection that transformed normally into mild within just seven days after the challenge, and could not depend on evaluating the activity of the phage preparations, whereas the traumatized bladder mucosa model resulted in the chronic infection that lasts for more than 30 days with just a little drop in the number of bacteria recovered from the urine culture or the homogenates of bladder and kidney. No systemic infection was developed through the course of mouse UTI (12).

The success of the treatment of mouse experimental model by intraperitoneal and transurethral administration of both single and phage cocktail preparations is indicated for the potential usage of those phages in the treatment of human UTI cases by parenteral injection in addition to other routes of phage administration. This property makes such treatments simple and easy for routine work just like chemotherapy (13-15).

The success of phage PEC80 in the treatment of mouse model of UTI induced by the host bacterium *E. coli* 80 and failure of the same phage in the 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; however, the success of the 25 phages cocktail in the treatment of mouse model of both strains was due to its broad-host-range against all *E. coli* isolates that obtained from the combination of various mechanisms of phage adherence and lysis to 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 (13).The importance of such preparation is not only concerned 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 the embryos, fetuses, 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*, such as enteropathogenic *E. coli*, enterotoxigenic *E. coli*, and enteroinvasive *E. coli*, which represents life-threatening conditions, especially, in the cases of multi-drug and pan-drug resistant strains (21).

4. Conclusion

In conclusion, the 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 host range made it possible to employ phage therapy as a potent, promising, and alternative therapy for the cases of UTIs. Furthermore, the success of the treatment of the mouse model of chronic UTI by the phage cocktail shows a strong indication for promising possibility of the phage cocktail in the treatment of UTIs via transurethral and intra-peritoneal routes. In addition, the simplicity and rapidity of phage therapy in the treatment of chronic UTI was a 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.

Authors' Contribution

Study concept and design: H. K. M.
Acquisition of data: H. K. M.
Analysis and interpretation of data: B. M. A.

Drafting of the manuscript: M. A. A.
Critical revision of the manuscript for important intellectual content: H. K. M.
Statistical analysis: S. A. G. K.
Administrative, technical, and material support: H. K. M.

Ethics

An animal model study was conducted under the recommendations of ethics of the animal model commission of the Iraq ministry of higher education and scientific research.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

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