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Phage Therapy for Treatment of UTI Caused by Multi-Drug-Resistant *E. coli* in Vitro and in Vivo Study

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Abstract

Background Multidrug-resistant (MDR) bacteria cause a substantial health burden. *Escherichia coli* (*E. coli*) is one of the most common causative agents of bacterial infections in urinary tract infection (UTI) and emergence of multidrug resistant *E. coli* is a major public health threat worldwide. In this respect, recent studies have proposed bacteriophage (phage) therapy as a potential alternative therapy to antibiotics for treating MDR bacteria.

Objective To evaluate the potential of bacteriophage therapy to treat a drug-resistant UTI caused by *E. coli* in rabbits.

Methods This study was carried out during the period from September 2019 to February 2021 in Kirkuk Province. A total of 30 bacterial isolates of *E. coli* were collected from bacteriology isolated - clinical specimens. Full diagnostic procedures were conducted to confirm the diagnosis of *E. coli*; moreover, antibiogram was done to identify MDR *E. coli* isolates. Lytic phages were isolated using phage amplification assays. Phage cocktail was prepared accordingly. Rabbit animals with induced UTI were orally fed with *E. coli* specific phage cocktail. Phages and *E. coli* bacteria were isolated from urine of rabbits for evaluating the success rate of phage therapy.

Results It was shown that four bacteria isolates did establish specific lytic phages for *E coli*. Orally-fed phages to *E. coli* were isolated from the urine of rabbits and peaked at the second day with continuous decline in titer over the third and fourth days after phage administration. The average titer plaque-forming unit/ml (PFU/ml) for *E. coli* specific phages was 46, 15 and 1 at the second, third and fourth days. Regarding the in vivo activity of phage on bacterial growth, the bacterial count in urine was shown to decline remarkably after 24 hr till 4 days after the oral administration of lytic phages. The decline in bacterial count in urine was highly significant and reached up to 7-8 log.

Conclusion Phage cocktail therapy was shown to be effective in treating uropathogenic MDR *E. coli* and bacteriophage therapy was safe during this study and no side effects were observed.

Keywords MDR, plaque-forming unit (PFU), colony-forming unit (CFU), phage therapy, *E. coli*, bacteriophage

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List of abbreviations: CFU = Colony-forming Unit, *E. coli* = *Escherichia coli*, XDR = Extensively drug-resistant, MDR = Multidrug-resistant, PFU = Plaque-forming unit, UTI = Urinary tract infection

Introduction

Urinary tract infections (UTI) is considered to be the second most common bacterial disease after pneumonia, mainly due to the high frequency

of period recurrences infections and lead to cause of septicemia [1,2]. The widespread and uncontrolled use of antibiotics during therapy and prophylaxis of UTIs results in the continuing increasing antibiotic resistance and emergence of multi-drug resistant (MDR) and extensively drug-resistant (XDR) uropathogens [3,4].

UTI is one of the most common reasons of morbidity and mortality in the elderly, accounting for 15.5% and 6.2% of hospitalization and deaths of people aged 65 years or older, respectively [5,6]. Women are more susceptible to infected than men due to behavioral factors, their anatomy, and practice such as use of diaphragm and spermicides [7]. Infections may affect women at any age, especially women with frequent sexual activities and women of childbearing age as well as old men and infant boys [8]. UTI is classified as either lower (confined to the bladder) or upper (pyelonephritis) and it can be clinically divided into two forms: uncomplicated and complicated. Uncomplicated form represents the conditions when patients exhibit a healthy status previous to the infection without non-catheterized, non-pregnant, and no structural abnormalities, on the other hand, UTI is reported as complicated when patients are immunocompromised or experience risk factors such as pregnancy and urinary retention [9,10].

Escherichia coli (*E. coli*) is the most common (80%) cause of the uncomplicated UTI and catheter associated infections. One of the main features of uropathogenic bacteria that makes them more resistant to the host immune system and chemical antibiotics is their capacity to form single or mixed-species biofilm [11,12].

The phage therapy the therapeutic use of bacteriophages for the treatment of pathogenic bacterial infections. Bacteriophages, are a form of virus that attach to bacterial cells and inject their genome into the cell, halting or distraction. The bacterial infection and bacteriophages are much more specific than antibiotics, they can be divided into two categories: monophage and polyphage therapy. Two or more phages mix and they cover various bacterial hosts in a single product as a phage cocktail, which is typically more effective in treating bacterial infections [12,13]. Furthermore, recent studies have reported that despite the presence of the different factors such as biofilms and other antibiotic resistance factors in UTI, phages along with other antimicrobial strategies may be effective in preventing and treating these infections by increasing the synergistic effect [14,15].

Therefore, phage therapy could be a useful choice in combating multidrug-resistant (MDR) uropathogenic bacteria such as *E. coli*. But it appears that the function of commercial phage cocktails to inhibit MDR bacteria is limited. This narrow activity could result from the absence of specific phages targeting contemporary MDR strains, which are spreading in different settings ([16,17]. Changes within the bacterial cell morphology empowered fast phage development and cell lysis. Besides, the set of phage lytic enzymes, particularly those responsible for local peptidoglycan degradation, could enhance antibiotic penetration through biofilm matrix.

On the other hand, phage cocktails can be mitigated when combined with antibiotics or phage cocktail along with polysaccharide-degrading enzyme, depolymerases, these approaches can be used for the treatment of *E. coli* biofilms present on urinary catheters, which is one of the major causes of UTI. In addition, overexpression of the efflux pump and autoinducer synthase that accelerate the synthesis and transport of acylated homoserine lactones can be related to the increase of biofilm formation. this activity may depend on the type of bacterial pathogen and the antibiotic used [18].

The objective of this study was to investigate the effect of phage therapy on Gram negative bacteria *E. coli* isolated from urine of patients suffering from urinary tract infection.

Methods

This study was carried out during the period from September 2019 to February 2021 in Kirkuk province to determine the phage therapy activity against thirty isolates of MDR *E. coli*, which were collected from urine samples of patients at Kirkuk General Hospital.

Full diagnostic procedures were conducted to confirm the diagnosis of *E. coli*; moreover, antibiogram was done to identify MDR- *E. coli* isolates. Lytic phages were isolated sewages using phage amplification assays. Phage cocktail was prepared accordingly. In these experiments, 8 albino rabbits' animals (6 rabbits for test group and 2 rabbits for control group) of mean body weight 2.02±0.30 Kg per rabbit used, and the age of these rabbits was 9-12 weeks. Control groups of rabbits were received bacteria.

The total 6 test group rabbits were introduced to the base line of 5 ml of 1.5 $x10^8$ colony forming unit per ml (CFU/ml) for bacterial infection by urinary tract catheter (6 mm). Then, every 4 hr, the rabbits were monitored for their health and physical activities and timely health score was recorded. After 24 hr, samples of urine were collected from each rabbit for bacterial isolation by using MacConkey's culture media. The rabbits were received orally 2.5 ml of $2.5x10^6$ plaque forming unit per ml (PFU/ml) of specific single phage. Later, each infected rabbit was received orally 7.5 ml of 7.5 x 10^6 PFU/ml of phage cocktail in order to evaluate phage therapy to treat UTI. Phages and *E. coli* bacteria were isolated from urine of rabbits for evaluating the success rate of phage therapy. Rabbits were then monitored for their health and physical activities and health score was recorded in addition to urine samples were collected by urinary catheter after 30 minutes (1, 2, 4, 6 and 24) hr daily for 4 days, for bacterial and phage isolation. Regarding the in vivo activity of phage on bacterial growth, the (CFU/ml) of isolated *E. coli* from the urine of rabbits, which phages orally-fed were the highest (CFU/ml) of bacteria count isolated at the first day and later at the second, third and fourth days were gradually decreased.

Titers of specific and lytic phages isolated and optimized to the studied bacterial isolates were obtained by using top layer plaque assay, which was used to further screen, amplify, and measure phages.

Results

Among 30 bacterial isolates of *E. coli*, after performing initial bacteriological and phage tests, it was shown that four bacteriophage isolates did establish specific lytic activity against isolates for *E. coli*, as shown in table 1.

Table 1. The plaque characteristics of isolated phages to MDR bacteria

Bacteriophage isolates	Plaques Size (mm)	Margin cut	Plaques clarity	Plaques shape
PEc1	5.5	regular	Clear	Circular
PEc2	2.6	regular	Clear	Oval
PEc3	3	Irregular	Semi-clear	Circular
PEc4	1.5	regular	Clear	Circular

PEc: Phage to *E. coli*

Table 2 shows the comparison of titer (PFU/ml) of single phages used to *E. coli* isolated from the urine of rabbits; the phage titer was detected 24-48 hr after administration and started to decline at the second day with continuous decrease over the third and fourth days. So, the average titer (PFU/ml) for *E. coli*

specific phages was about 46, 15 and 1 at the second, third and fourth days, respectively.
As shown in table 3, the present study revealed that after using phage cocktail for infected rabbits with *E. coli*, the phage cocktail that isolated from the urine of these rabbits dropped gradually from the second to the seventh day and ranged from 173.3 to 6 (PFU/ml).

Table 2. Comparison of (PFU/ml) of single phages used *E. coli* isolated from urine

Phage single to *E. coli* isolates	Phage single (PFU/ml) in urine of rabbits		
	Day 2	Day 3	Day 4
$E1P_S$	64	23	3
$E2P_S$	46	15	1
$E3P_S$	28	8	0

EP_s: Phage single to *E. coli*, PFU: Plaque forming unit

Table 3. Comparison of (PFU/ml) in urine of rabbits isolated phages cocktail

Phage cocktail to *E. coli* isolates	Phage cocktail (PFU/ml) In urine of rabbits						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
E_1P_K	156	184	175	170	165	72	32
E_2P_K	80	210	136	98	85	33	10
E_3P_K	173.3	200	60	56	44	12	7
E_4P_K	118.6	60	50	40	15	8	6

EP_k: Phage cocktail to *E coli*, PFU: plaque forming unit

Regarding the in vivo activity of phage on treating UTI in rabbit animals, the bacterial count (CFU/ml) of *E. coli* isolated bacteria from the urine of rabbits that fed orally with specific phages declined remarkably as shown in table 4.

Table 4. Daily estimation of bacterial count (CFU/ml) of *E. coli* in urine of rabbits treated with specific lytic phages

Days	Bacterial (CFU/ml) with single dose of specific phage
	E1-3Ps
Day 1	6,700,000,000
Day 2	430000
Day 3	246.6
Day 4	0.66

CFU: Colony forming unit

Number of colony (CFU/ml) of *E. coli* in urine of rabbits without phages as control groups; in regard to UTI-induced rabbits with *E. coli*, as shown in table 5 were orally administered for

monitoring the bacteria colonizing the urinary tract of rabbit animals. The titer (CFU/ml) of bacteria isolated from the urine from day 8 to day 21 showed slightly variation with remain closely related titers of bacterial colonies in compare with rabbits induced specific phages after bacterial infection.

Table 5. Titer (CFU/ml) of E. coli in urine of rabbits without phages

Control group (*E. coli*)	Colony Number (CFU/ml)							
	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 21
CGE1	296 × 10^6	293 × 10^6	298 × 10^5	290 × 10^6	294 × 10^6	289 × 10^6	290 × 10^6	273 × 10^6
CGE2	295 × 10^6	296 × 10^6	290 × 10^6	288 × 10^6	291 × 10^6	289 × 10^6	284 × 10^6	280 × 10^6

CGE: Control Group *E. coli*

Discussion

The present study revealed that after using phage cocktail for infected rabbits with single bacteria including *E. coli*, were the (PFU/ml) of phage cocktail that isolated from the urine of these rabbits dropped gradually from the second to the seventh day with ranging from 173.3 to 6 (PFU/ml) for *E. coli*.

The antibacterial activity of coliphages were assessed against 15 multiple antibiotic resistant E. coli isolates obtained from UTI. All isolates (100%) tested were sensitive to bacteriophge lysis effect. This indicated that bacteriophge activity as anti-bacterial therapy was specifically effective against E. coli isolates.

Titers of specific and lytic phages isolated and optimized to the studied bacterial isolates were obtained by using top layer plaque assay, which was used to further screen, amplify, and measure phages. Data were collected by visual examination of the plates and by manual plaques counting. The titers of the isolated and optimized phages were ranged 1.8 x 10^6 - 2.6 x 10^{11} for *E coli*. The comparison of (PFU/ml) of single phages used *E. coli* isolated from the urine of rabbits, were declining the (PFU/ml) of specific phages at the second day with continues decreasing the (PFU/ml) gradually at the third and fourth day.

Regarding the in vivo activity of phage on bacterial growth, the (CFU/ml) of isolated bacteria from the urine of rabbits received phages orally-fed were the highest (CFU/ml) of bacteria count isolated at the first day and later at the second, third and fourth days were gradually decreased. These results were obtained through carefully designed and conducted experiments

The pharmacology of phages necessitates the study of interactions between phages and bacteria as well as interactions between phages and body tissues [14]. Successful and safe phage therapy involves the elective control of phage–host interactions involving two fundamental components: pharmacodynamics and pharmacokinetics. Therapy for treating UTI is one of the most promising applications for phages and one of the few that have been studied in a multi-stage clinical trial [15].

In conclusion, bacteriophage single and cocktail for *E. coli* can be prepared in laboratory with simple measures; they were feasible, time saving, flexible and economic. Formed phage cocktails were useful to tackle the problem of bacterial resistance to some individual phages. Phage therapy is safe when used in rabbits and considered as a practical tool for starting ready-made therapeutic phage preparations for treating bacterial infections.

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Author contribution

Dr. Abdel-Ameer: designed the research and conducted study analysis. Dr. Saqi : conducted the research.

Conflict of interest

There is no conflict of interest among authors of this manuscript.

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