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Bacteriophage therapy for treating multiple-drug resistant urinary tract pathogen, *Escherichia coli*

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ABSTRACT

The aim of the study is to prove the efficacy of phage therapy for treating multi drug Resistant (MDR) *Escherichia coli* isolate from urine sample of UTI patient. The clinical isolates of *E.coli* was obtained from 5 out of 10 urine samples of Urinary Tract Infection (UTI) patients using Hicrome UTI agar. The isolate was checked for their resistance, by using antibiotic susceptibility test. The multidrug resistant (MDR) isolate was further subjected to phage susceptibility test using lambda phage and phages isolated from sewage. The lambda phage and phages isolated from sewage was found highly lytic to MDR *E.coli*. Phage therapy can be used for treating MDR *E. coli* and can even compensate the progressively failing antibiotics.

Keywords: Urinary Tract Infection, Multidrug Resistant *E.coli*, Bacteriophage, Phage therapy

INTRODUCTION

Escherichia coli is a gram negative, aerobic or facultative anaerobic, rod-shaped and coliform bacterium. It is motile by peritrichous flagella, though some strains may non-motile. Capsules and fimbriae are found in some strains.

Urinary tract infection (UTIs) involves the infection of the urinary tract which includes the urethra, bladder, ureters or the kidneys. According to a 2012 report in the Journal Emerging Infectious Diseases, 85 per cent UTI is caused by *E.coli* and other coliforms. Other common bacteria that can cause UTI are *Staphylococcus saprophyticus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* Symptoms of UTIs include urinary frequency, painful urination, dysuria, haematuria, foul-smelling and cloudy urine, suprapubic or loin pain, pyrexia, nausea and vomiting, burning sensation and abdominal pain.

Urinary tract infection is one of the most common bacterial infections that act as a threat to human health with increasing resistance to antibiotics. It has been found in different studies from various parts of India have shown occurrence of increased rates of antimicrobial resistance among *E coli.* UTI due to multi drug resistant (MDR) *E.coli* increases the cost of treatment, morbidity and mortality especially in developing countries like India (Niranjan and Malini, 2014).

The wide use of antibiotics has made all these urinary tract pathogens as resistant strains so, there is an urgent need of an alternative therapy for treating these multiple drug resistant pathogens. The alternative therapy for UTI is bacteriophage or phage therapy. Bacteriophages or phages are bacterial viruses that invade bacterial cells and lyse it. According to recent report by WHO, in the US 14,000 people die each year from hospital acquired drug-resistant infections, and worldwide it has been found that 60% of such infections are drug-resistant.

Advantages of phage therapy includes that they are highly effective in killing their targeted bacteria i.e., their action is bactericidal, they multiply at the site of the infection until there are no more bacteria, there are no side effects and it is highly species specific i.e., it kills the particular pathogenic bacteria without affecting the normal flora. Despite all these advantages there are also certain disadvantages which can't be neglected, phages can be rapidly cleared by spleen, liver and other filtering organs of reticuloendothelial system. This problem can be overcome by serial passaging in mice which modifies the viral coat proteins and makes the phage mutant, which helps the reticuloendothelial system not to recognize the phages as foreign particle and result in their lysis.

MATERIAL AND METHODS

Sample collection

Urine samples from patients suffering from urinary tract infection were collected from a laboratory. A total of ten samples were collected. The samples were mostly collected from females of age 35-50 years of age.

Isolation and processing of urinary tract pathogen

The urine samples were processed by streaking with a sterile loop on a selective media, Hicrome UTI agar. Then the inoculated plates were incubated at 37°C for 24 hours. After incubation, the plates were observed for colonies. The identification of *E.coli* was later confirmed by inoculating the colonies obtained on Hicrome UTI agar on MacConkey agar and biochemicals and then incubated at 37°C for 24 hours.

Antibiotic susceptibility test

The antibiotic susceptibility test was carried out to determine Multiple-Drug Resistant *E.coli* by Kirby Bauer Disc Diffusion Method using Mueller Hinton agar. The isolated *E. coli* were cultivated in Muller Hinton agar medium and then the commercially prepared antibiotic disks were placed in the agar surface using sterile forceps, and pressed gently to ensure full contact with the surface of the culture medium. The plates were then incubated at 37°C for 24 h. The antibiotics used were: Ampicillin (AMP), Rifampicin (RIF), Streptomycin (S), Tetracycline (TE), Ciprofloxacin (CIP), Gentamycin (GEN), Trimethoprim (TR), Sulfamethoxazole (SM), Cefuroxime (CXM) and ceftriaxone (CTR).

Plaque assay

The effect of bacteriophage (lambda phage and phages isolated from sewage) on the isolated multiple drug resistant *E.coli* was performed by plaque assay method.

Lambda phage

Serial dilution of the lambda phage stock was done using nutrient peptone broth (10^{-1} to 10^{-6} and one control without phage stock). A tube marked as seven was used to serve as control (which contains only 1 ml of media and no phage). Aliquotes of *E.coli culture* was added to each tube of phage (about 60 µl). The first two dilutions (tubes 1 and 2) were not plated, so those two dilutions were then set aside. Using aseptic technique, 4 ml of tryptone top agar was added to the control tube and the other tubes (tube 3 to tube 6), and then immediately the mixture was poured onto the tryptone base-layer agar plate. To avoid contamination, plating was started from the most diluted phage samples to the less diluted ones (it was started from tube 6 and proceeded till tube 3). After the plates set, the plates were inverted and was placed in a 37°C incubator for 24hrs. After incubation, the plaques on the plates were observed.

Phages isolated from sewage

The number of phage virions in a raw sewage sample was increased by enrichment process. For this, 5ml of Deca strength phage broth was taken in a sterile conical flask. To that 5 ml of the multidrug resistant *E.coli*

isolate suspended in BHI broth and 45 ml of raw sewage was added. (The Deca strength media is ten times as strong as ordinary broth to accommodate dilution with 45 ml of sewage). Then the mixture was incubated at 37°C for 24 hours. The phage from the multidrug resistant *E.coli* in the enrichment medium was separated by membrane filter technique using a filter having a pore size of 0.45µm. This filter hold backs the bacteria and allows only the phage virions to pass through. This process is called as filtration method. This method was performed by centrifuging the sewage *E.coli* mixture at 2500 rpm at 10 mins. Then the supernatant was filtered through a millipore filter. Then the filtrate was aseptically transferred to a sterile conical flask. Evidence of the phage in the filtrate was produced by seeded agar overlay method. Soft nutrient agar tubes were prepared and to each tube immediately 0.1ml and 0.01 ml of the filtrate was added. Then to the tubes 1ml of the multidrug resistant *E.coli* suspended in BHI broth was added. The contents were mixed thoroughly for about 10 secs by rolling the tubes between the palms. Bubbles were not allowed to introduce into the agar (because bubbles in the agar overlay will look like small, clear plaque). The seeded agar overlay was then poured over the hard agar overlay, nutrient agar. Then the plates were incubated at 37°C for 24hrs. After incubation, the plates were examined for plaques.

RESULTS AND DISCUSSION

Isolation of organism

A total of 10 urine samples were processed for isolation of *E.coli* using Hicrome UTI agar. Pinkish purple colonies indicating *E.coli* were isolated from 5 urine samples which shows that growth was present in 50% samples whereas 40% samples had no *E.coli* isolates (Fig:1). The colonies obtained when streaked on MacConkey agar (Fig: 2) and biochemicals for further confirmation showed pink colour lactose fermenting colonies and IMViC (+ +- -.) With the above study we can therefore also infer that *E.coli* is the most potential urinary tract infection causing organism.

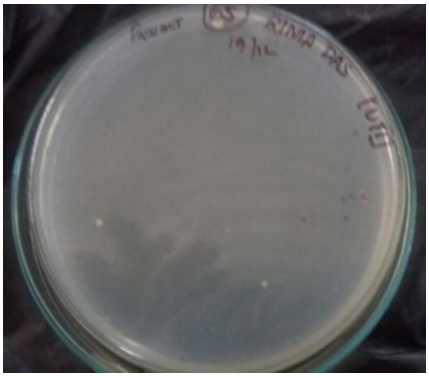


Fig 1: Pink purple colonies of *E.coli* on Hicrome UTI agar.

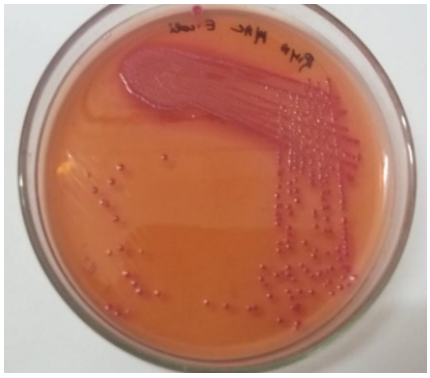
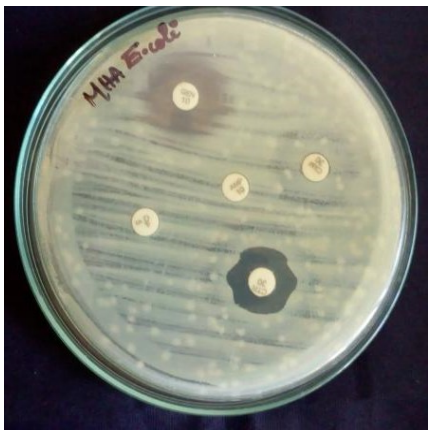


Fig 2: Pink lactose fermenting colonies of *E.coli* on MacConkey agar.



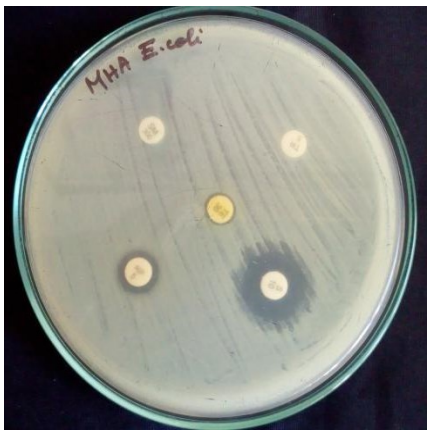


Fig 3 and 4 : Antibiotic sensitivity test showing MDR of *E.coli*

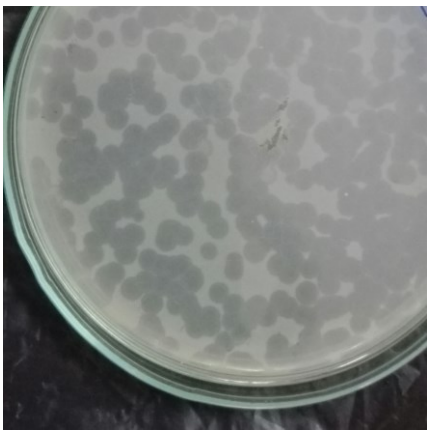


Fig 5: Plaques by phages isolated from sewage.

Table 1: Antimicrobial drug resistance of *E.coli* isolates

Drug name	Drug conc.	S1 (mm)	S2 (mm)	S3 (mm)	S4 (mm)	S5 (mm)	ATCC (mm)
SM	300mcg	2.7 (S)	2.6(S)	2.8(S)	- (R)	2.9(S)	2.7(S)
TR	5mcg	2.5(S)	2.7(S)	3(S)	- (R)	3(S)	2.5(S)
GEN	10mcg	1.4(I)	1.7(S)	1.9(S)	1.5 (R)	1.6(S)	1.2(R)
CXM	30mcg	- (R)	2.2(S)	2.2(S)	- (R)	2.3(S)	- (R)
CIP	5mcg	- (R)	1.5(R)	1.3(R)	1.3(R)	1.4(R)	- (R)
S	10mcg	1.6(S)	1.2(R)	1.5(R)	1.8(I)	2(S)	1.8(I)
TE	30mcg	2.3(S)	1.6(I)	2.2(S)	- (R)	1(R)	1.1(R)
CTR	30mcg	- (R)	2.3(S)	3(S)	1.5(R)	2(S)	- (R)
AMP	10mcg	- (R)	- (R)	- (R)	- (R)	- (R)	-(R)
RIF	5mcg	1.2(R)	- (R)	1 (R)	1(R)	1.1(R)	1.2(R)

Antibiotic sensitivity test

Antibiotic sensitivity testing was carried out on the 5 isolates of *E.coli* using Kirby Bauer Disc Diffusion Method. Zone of inhibition for 10 antibiotics were noted (Table: 1) and it was marked as: S- sensitive, R- resistant and I- intermediate (by referring the standard antibiotic sensitivity chart). Out of 5 isolates, one of the isolate was found to be resistant to 9 different types of antibiotics (out of 10) while the other isolates were also comparatively resistant (Fig: 3). And hence, this strain of *E.coli* was considered to be multidrug resistant strain.

The S1, S2, S3, S4 and S5 in the Table represent the different isolates of *E.coli* obtained from urine of UTI patients. Here we can observe from the table that the *E.coli* isolate 4 is a multidrug resistant (MDR) strain.

Plaque assay

The multidrug resistant *E.coli* was subjected to phages and the following results were obtained:

Lamda phage

In this study lamda phage active against *E.coli* was used and visible plaques were observed on the plate when tested on bacterial lawn of specific MDR *E.coli.*

Phages isolated from sewage

In this test few phages active against *E.coli* were isolated. All of the isolated phages formed visible plaques in the early stage when tested on bacterial lawn of specific MDR *E.coli* (Fig: 4 and Fig: 5).

The above two results with different kind of phages proves the efficacy of phage therapy multidrug treating drug resistant urinary tract pathogen *E.coli.*

As proved in this study that *E.coli* was found to be the most frequent causative agent of UTIs. Many studies have also synchronize with the results of this study like diversity of urinary tract pathogens and drug resistant isolates of *Escherichia coli* in different age and gender groups of Pakistanis. This above study by Bashir *et al*., (2008) has also showed increased resistance of *E.coli* to ampicillin, clotrimoxazole, ciprofloxacin and gentamycin which also goes in harmony with this study. Because this study also has proved that the most of *E.coli* isolates from UTI patient's urine sample are resistant to *ampicillin, clotrimoxazole, ciprofloxacin and gentamycin*.

The antibiotic susceptibility pattern of *E.coli* isolates has also revealed that most of these isolates are resistant to one or more antibiotics and it's resistance is increasing day by day. This agrees with several previous studies conducted on screening of multidrug resistant *E.coli* from urinary tract infected patients (Jemimah *et al*., 2012) and antimicrobial resistance pattern in *Escherichia coli* causing urinary tract infection among inpatients(Niranjan and Malini, 2014).

The current study also showed the efficacy of single phage as well as multiple phages (isolated from sewage) to treat multidrug resistant urinary tract pathogens. The finding of the study is also in complete harmony with the report stating bacteriophages as potential treatment for urinary tract pathogens (Wilbert *et al*., 2016).

CONCLUSIONS

UTI remains as a threat to human health. The bacteria mostly causing the UTI is *E.coli* (as found in this study). The drug of choice in the treatment of UTI has become narrow today due to the increased resistance that the common UTI pathogens show to drugs which have been used previously.

Multidrug-resistant bacteria have opened a new window of treating UTI by phage therapy. As phages lyse only specific pathogens without disturbing normal bacterial flora and it also pose no risk to anything other than their specific bacterial host so, efficacy of phage therapy appears to be very safe.

Conflicts of interest: Not declared

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