Pak Heart J 2023:56(03)
ISSN: 0048-2706 (Print), ISSN: 2227-9199 (Online)

Isolation of bacteriophage from sewage, Pond, Lake Water against MDR Escherichia coli

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Abstract

Background: The prevalence of highly resistant pathogens has created significant challenges in the treatment of patients, particularly within hospital settings. The practice of phage therapy, which uses bacterial viruses (phages) to treat bacterial infections, has been around for almost a century. The universal decline in the effectiveness of antibiotics has generated renewed interest in revisiting this practice. **Method:** he study was conducted at the Department of Microbiology, RKDF Medical College Hospital & Research Centre, SRK University, located in Bhopal, Madhya Pradesh. **Results:** We included total 140 cases. Among the 140 Escherichia coli, 122 were MDR Escherichia coli and rest were normal E. coli. After that, Bacteriophages were prepared against 122 MDR Escherichia Coli which were isolated from sewage pond lake water and see the in vitro lytic efficacy of bacteriophages for their presence. So the presence of bacteriophage is more in sewage water as compared to other water samples. **Conclusion:** In conclusion, it may be suggested bacteriophage can be used as a therapy and phage therapy might be beneficial for the patient who are suffering with the MDR strains from a long period of time.

Keywords: Lytic activity of bacteriophage, AST, MDR.

Introduction

Escherichia coli is predominantly found colonizing the intestinal tracts of mammals.[1] E. coli have been discovered to rapidly colonize the gastrointestinal tract (GIT) of infants shortly after birth. Additionally, studies have reported that E. coli can be beneficial to the host.[2] It has been found to be excreted in the feces and to survive in the atmosphere. Due to its prevalence in the gastrointestinal tract and its association with fecal contamination, Escherichia coli have been widely recognized as an indicator organism for assessing food and water hygiene.

More than a hundred years ago, the discovery of bacteriophages was made. Ernest Hanbury Hankin, a British bacteriologist born in 1865 and passed away in 1939, made a notable discovery in 1896. He documented the unexpected antibacterial properties of river waters in India against cholera. Additionally, unique characteristic was retained even after the water was passed through an extremely fine porcelain filter. (Hankin, 1896).[3]

Despite the discovery made by Hankin, he did not pursue it any further. The First Medical Experiments using Phages were Conducted by d'Herelle after his significant investigation into phages.

According to E.V. Orlova, in 1917 (d'Herelle), successfully treated a young boy suffering from severe dysentery by administering phages (d'Herelle, 1917).[4]

Before the discovery of penicillin, d'Herelle and a group of scientists from Georgia (formerly part of the USSR) founded an institute dedicated to researching the characteristics of bacteriophages and their potential for the treatment of bacterial infections, ten years in advance. Insufficient comprehension of the fundamental aspects of phage biology and the intricate arrangement of their molecular components has been a contributing factor to specific clinical challenges and limitations. Discovery of antimicrobial drug in the late 1930s was a major breakthrough in medicine, leading to a decline in research on the medical applications of phages. However, the excessive use of antibiotics has resulted in the rise of bacterial resistance, diminishing the efficacy of these drugs in combating infections. The prevalence of highly resistant pathogens has created significant challenges in the treatment of patients, particularly within hospital settings.[5-6] So the aim of this study is Isolation of bacteriophage from sewage, Pond, Lake Water against MDR Escherichia coli.

Materials and Methods

Study Period

The study period of this study was over a two years.

Study Area

This study was conducted in Department of Microbiology, RKDF Medical College Hospital & Research Centre, SRK University, located in Bhopal, Madhya Pradesh.

Data Collection

We collected 122 strains of Escherichia coli that were resistant to multiple drugs from various clinical samples, using antibiotic susceptibility patterns as the selection criteria. Subsequently, we targeted multi-drug resistant E. coli with bacteriophages, obtained from sources such as lakes, sewage, and ponds, using their ability to cause lysis as the basis for selection. The process for acquiring these bacteriophages began with water collection, followed by decontamination steps. Afterward, we cultured the bacteria on Müller Hinton agar and introduced the bacteriophages onto the agar surface. By observing lytic activity, we could confirm the presence of the bacteriophages.[7]

Data analysis.

Results

Out of the total of 140 Escherichia coli strains, 122 were identified as multi-drug resistant (MDR) strains. These strains were isolated from a range of clinical specimens, including fluids, blood, pus, urine, and swab samples. For the isolation of bacteriophages, we examined a total of 9 water samples collected from different sources such as lakes, ponds, and sewage. Notably, we observed a higher prevalence of bacteriophages in the sewage water sample as compared to other water samples.

Table 1: Distribution of cases according to Escherichia coli

Isolates	No.	%
Normal E. coli	18	12.8%
MDR E. coli	122	87.2%
Total	140	100%

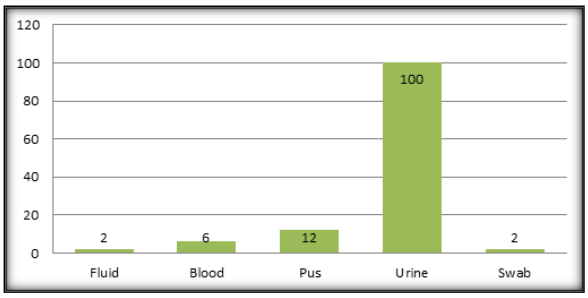

Chart:1 Distribution of Sample according to clinical specimens

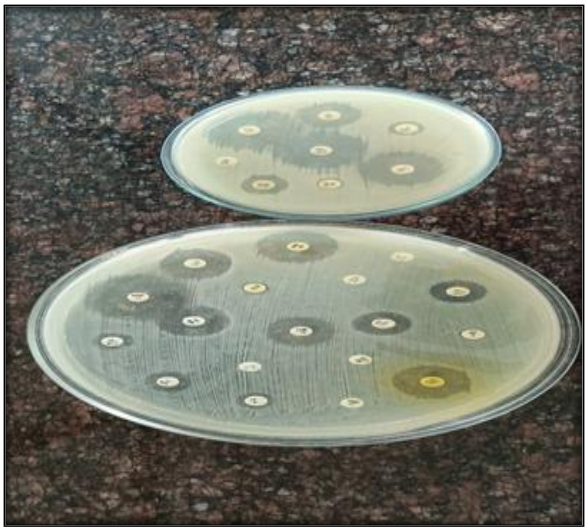

Figure :1 Antimicrobial susceptibility test

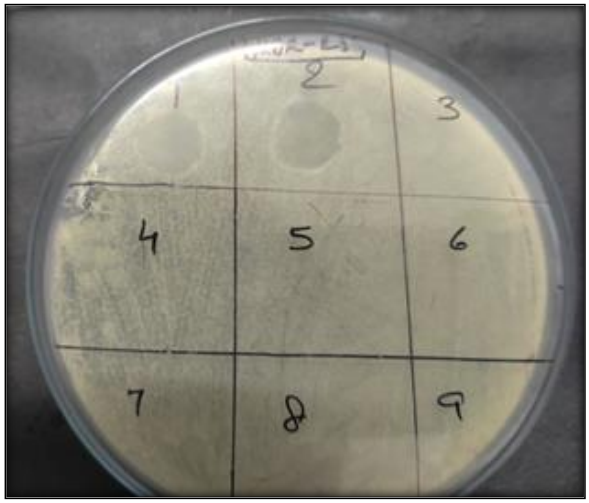

Figure: 2 Bacteriophage

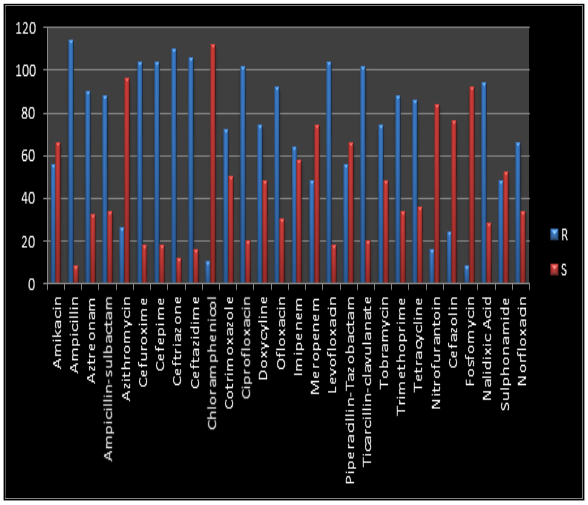

Chart :2 Resistant and susceptibility pattern of antibiotics of MDR Escherichia coli

Table: 2 Distribution of water sample

SR NO.	Water sample	No.
1	Sewage	3
2	Pond	3
3	Lake	3
Total		9

Table:3 Distribution of sample according to lytic activity

Sample source	Sample no.	Observed lytic activity
Sewage	1	66
	2	70
	3	42
Pond	4	20
	5	24
	6	4

Lake	7	2
	8	8
	9	0

Discussion

Escherichia coli are a normal flora of human intestine. It is recognized as a marker of fecal contamination in food .It is responsible for causing nosocomial infections in humans.[8] As an intestinal parasite found in both humans and animals, Escherichia coliis a prevalent pathogen known to cause a variety of complications in humans, including UTI, bloodstream infections, wounds infection, otitis media, and other related conditions.[9-11] The issue of antimicrobial resistance in urinary tract infections is a constantly evolving and concerning problem, with particular emphasis on the rising incidence Escherichia coli infection that exhibit frequent resistance to beta-lactams and other antibiotics.[12-13]

Our study's findings indicated that MDR Escherichia coli had a prevalence rate of 87.2%.There are a number of reason of these results which are as follow; irrational use of antibiotics, easy availability of antibiotic on the counter, take incomplete dose of antibiotic & not following the antimicrobial stewardship.

The present study finding has been supported by Ibrahim ME et al, Ibrahim, D.R, Jain, P. et al.[14-17]

The samples of this study have taken 9 water samples for the isolation of bacteriophages. Out of which, 3 water samples from the sewage,3 water samples from the pond, 3 from the lake. Significantly, we noted an elevated occurrence of bacteriophages in the sewage water sample in comparison to the other water samples. A.A. Abou Zeid conducted a study on the efficacy of bacteriophages against MDR Escherichia coli. The researchers collected water samples from various sources, including hospital wastewater, agricultural drainage water and sewage water, to isolate bacteriophages against E. coli. By using spot test and plaque assay they detected bacteriophages and E coli used as a host.[18]

The study by H. Brussow et al.[19] involved collecting water samples from various sources, such as sewer, wastewater, and rivers. They also included human and animal waste, for the isolation of bacteriophages. This is comparable to the approach taken in our study.[20]

Conclusions

In conclusion, it may be suggested bacteriophage therapy might be beneficial for the patient who are suffering with the MDR strains from a long period of time.

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