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ISOLATION OF BACTERIOPHAGE FROM SEWAGE WATER AND ITS ANTIMICROBIAL ACTIVITY AGAINST AMR BACTERIA

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ABSTRACT

Antibiotic resistance is a critical public health issue that affects people all over the world. Bacteriophage (phage) therapy, as an alternative to antibiotics, is one of the most effective ways to combat antibiotic resistance. For phage therapy bacteriophage must be both isolated from the environment and show lytic activity against target bacterial pathogens. A single phage or a mixture of phages can also be employed in phage therapy; mixed phages are more successful than single phages at lowering the number and/or activity of harmful bacteria. we aimed to use mixed phages against three AMR bacteria (*Pseudomonas aeruginosa, Escherichia coli,* and *Serratia marcescens*). Frequently first isolation of phages is done and then characterization, to isolate the phage alteration of the procedure is possible to get the desirable results. Isolated mixed phages were able to completely lyse bacterial cells and they are effective on all three AMR bacteria. These isolated mixed phages may be a good substitution to bacteria that are resistant to antibiotics and can also use in phage therapy.

Keywords: Bacteriophage isolation, phage therapy, AMR bacteria, antimicrobial activity

1. INTRODUCTION

Bacteriophage, also known as phage, is a virus-infecting and killing the bacteria. The global population of phage particles is estimated to be around 10^{31} virions **[1]**. A scientist name Félix d'Hérelle is a French–Canadian microbiologist, 1917 has recovered a *Shigella* as an antibacterial and he has coined the term "Bacteriophage" — means bacteria-killing **[2]**. After the discovery of bacteriophages in the early twentieth century, several researchers speculated on their (phages') ability to kill bacteria, implying that they could be used as therapeutic agents **[3]**. Phages typically infect and kill a single strain or line of bacteria, which limits their ability to infect all types of bacteria. This particular nature is very beneficial in the clinical setting, as only pathogenic bacteria are attacked, infected, and killed, leaving the guests alone with harmless and potentially useful bacteria **[4, 5]**. The capsid is attached to the tail with fibres that allow it to attach to receptors on the surface of bacterial cells. Most phages, except filamentous phages, have polyhedral capsids. Based on capsid composition, gene composition, and chemical structure bacteriophages are further divided. Bacteriophages are divided into 13 families Shown in **Figure 1 [6]**.

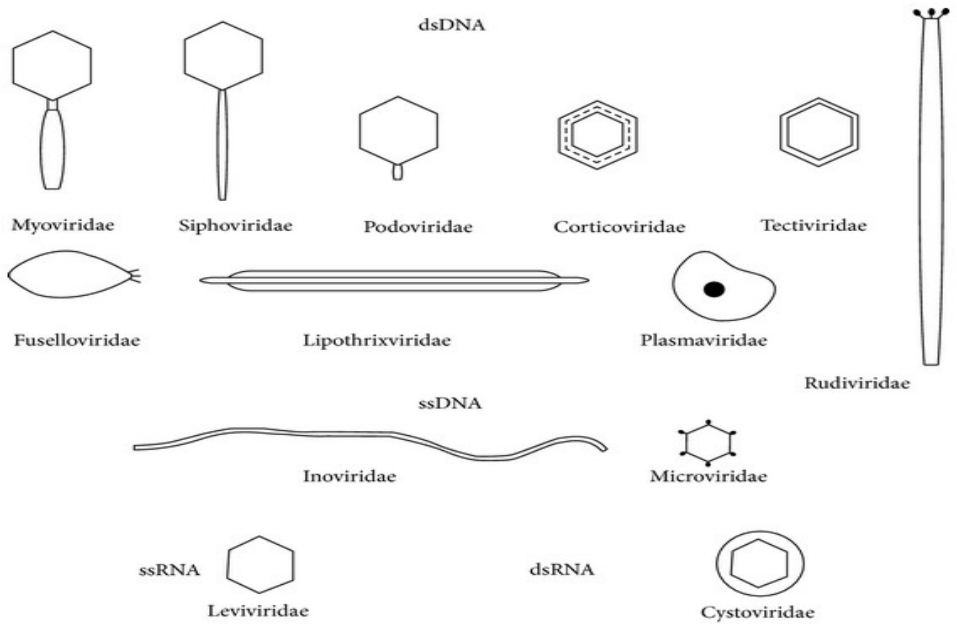

Figure 1: Schematic representation of major groups of bacteriophages. Reproduced with permission [7]

The genetic material of the phage takes control of the host's biosynthetic machinery. Inside the bacterial cell, different proteins are produced, including the outer protein,

which helps in the making of the capsid, and lysis proteins, which cause the host cell to lyse **[8]**.

Antibiotic resistance has increased and reduced the use of antibiotics. Between1960 and 1980 antibiotic resistance increased which resulted in making novel antibiotics but only a small amount of them developed with low resistance **[9]**. Due to the challenges and increased costs of treating antibiotic-resistant infections, antimicrobial resistance has become a global public health concern **[10, 11]**. This has increased the interest in alternate and conventional ways to control multi-drug-resistant bacteria. Lytic phages are preferred in making drugs because they are highly specific and do not have any harmful effects and do not disturb the normal flora **[12]**.

We aim to target three AMR bacteria bacteria (*Pseudomonas aeruginosa, Escherichia coli,* and *Serratia marcescens*). As per WHO *Escherichia coli* causes 6300 annual deaths **[13]**. Because this is found in human feces in high concentration and when these fecal mixes with drainage were already misused antibiotics are discarded as clinical aspects and produce resistance to microbial strain. *Pseudomonas aeruginosa* is the 3rd leading cause of nosocomial urinary tract infections (UTIs) **[14]**. *P. aeruginosa* is antibiotic resistant because of its ability to form a biofilm which creates a barrier for antibiotics to enter. *Serratia marcescens* also causes nosocomial infections, especially in neonatal intensive care units (NICU) **[15, 16]**. Therefore, our study aims to conduct the isolation of bacteriophages that show lytic activity against these AMR bacteria.

2. MATERIALS AND METHODS

Bacterial strain (*Pseudomonas aeruginosa, Escherichia coli and Serratia marcescens*), sewage water samples as a source of bacteriophage, Nutrient agar, nutrient broth and EMB agar, autoclave, incubator, glassware's (petri dishes, conical flasks, beakers, glass rods), laminar air flow, refrigerated centrifuge

2.1. SAMPLE COLLECTION

Water sample as a source of bacteriophage was collected from the sewage that is located near Waghodia bridge, Vadodara, Gujarat, India. Water samples (200 ml) were collected into sterile bottles, kept at a temperature of 4 °C, taken to the laboratory, and processed on the same day of collection.

2.2. HOST BACTERIA

Pure cultures of *Escherichia coli, Serratia marcescens, and Pseudomonas aeruginosa* were collected from the college laboratory itself and grown on the selective media, and further used as the primary host for the isolation of bacteriophage.

2.3. PROCESSING OF SEWAGE WATER SAMPLE

The samples were centrifuged at 4000 rpm for 15 minutes after being maintained at

37°C for 30 minutes. The supernatant was collected with a micropipette and spun in a chilled centrifuge at 7000 rpm for 15 minutes (Remi C-24). The supernatant was then evaluated for lytic activity against *P.aeruginosa* and *E. coli*.

2.4. ISOLATION OF BACTERIOPHAGE

To isolate bacteriophages against the host bacterial strain, the phage enrichment approach was applied (*E. coli*). Briefly, 15 ml of processed water sample was added to 2 ml of overnight developed bacterial cultures (host bacteria), and the mixture was incubated at 37°C for 24 hours in a shaking incubator (150 rpm). The mixture was then centrifuged for 20 minutes at 5000 rpm in chloroform, with the supernatant collected in a sterile eppendorf tube. To improve phage adsorption onto the bacterial surface, double agar layer method is performed by adding 300µl of the filtrate phage sample combined with 200µl of 6 hours hold bacterial culture (*E.coli*) in a sterile vial, along with 1M $MgCl_2$ and 20% maltose solution, shaken gently for 20 minutes before being introduced to a test tube containing 3ml of molten soft agar kept at 45°C in a water bath. After rotating the mixture it was put onto nutrient agar basal plates, allowed to solidify and then incubated at 37°C for up to 48 hours. A susceptible result was indicated by the formation of a clear or turbid plaque on the surface of the agar.

2.5. LYTIC ACTIVITY OF PHAGE

The lytic activity of phages can be measured using a spot assay, which is performed as follows: 5 ml of soft agar was mixed with 100µl of overnight bacterial culture, gently vortexed and then poured onto the surface of nutrient agar medium. The hard NA plates were infected with single drops of phage suspension and incubated overnight at 28°C. A susceptible result was indicated by the formation of a clear or turbid plaque on the surface of the agar.

3. RESULT

3.1. HOST BACTERIA

Collected pure cultures of host bacteria were grown on selective media to avoid any kind of contamination **(Figure 2)**.

3.2. ISOLATION OF BACTERIOPHAGE

The concentration of phages was high in sewage water and lytic activity was observed on plates by double agar overlay method against antimicrobial resistance (AMR) bacteria *Escherichia coli, Serratia marcescens* **(Figure 3-5)**.

3.3. HOST RANGE DETERMINATION

Spot assay is done to check the ability of phage to completely lyse the respective AMR bacteria. Isolated phage has shown lytic activity against AMR bacteria which are *Pseudomonas aeruginosa, Escherichia coli,* and *Serratia marcescens.* Lytic activity is observed with turbid spot-on bacterial lawn as shown in the **Figure 6, 7** below.

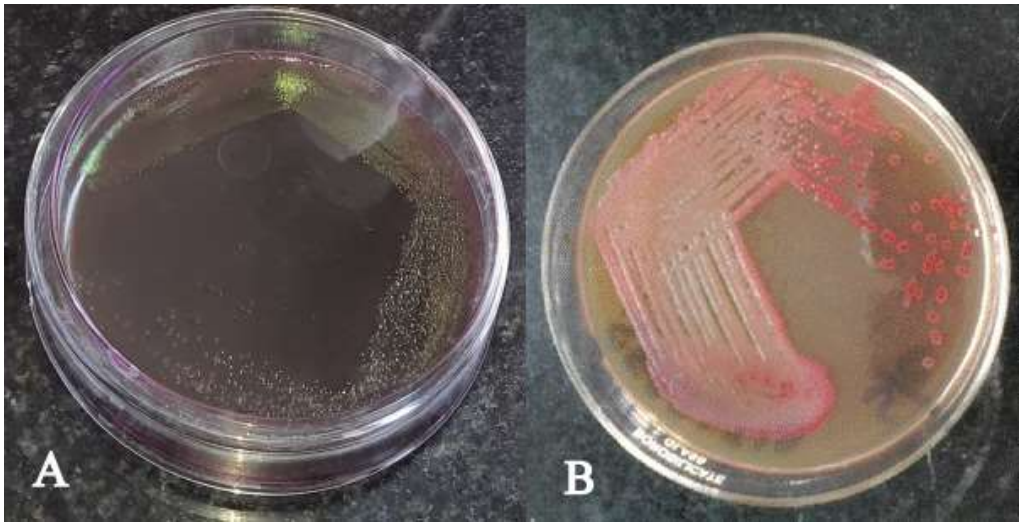

Figure 2: A). *Escherichia coli* on EMB agar B). *Serratia marcescens* on Nutrient agar

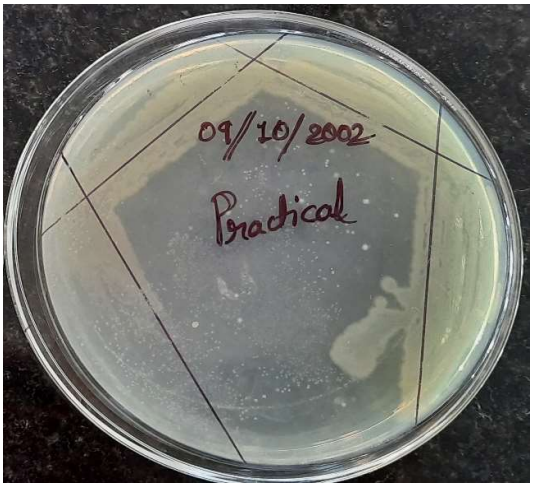

Figure 3: *Pseudomonas aeruginosa* on Nutrient agar

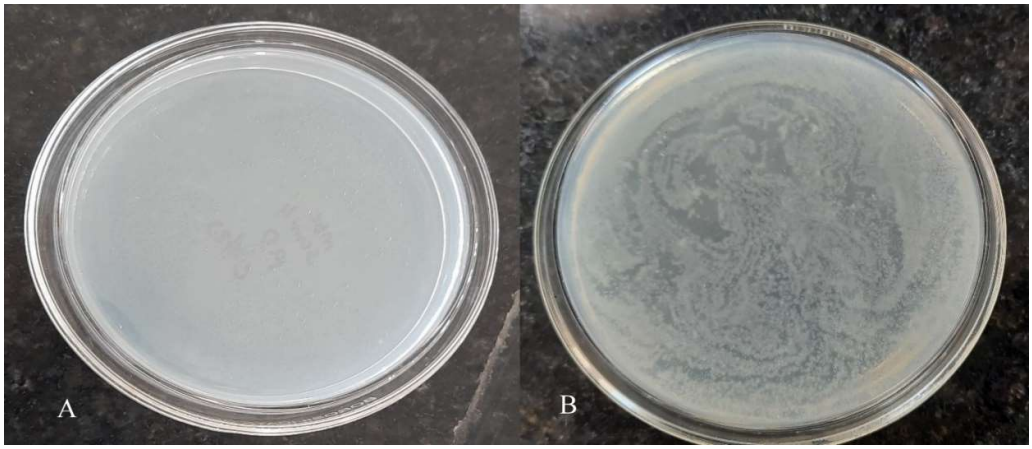

Figure 4: (A) Control, (B) Large and Small-sized clear plagues on *Escherichia coli*

Figure 5: Small-sized plagues on *Serratia marcescens*

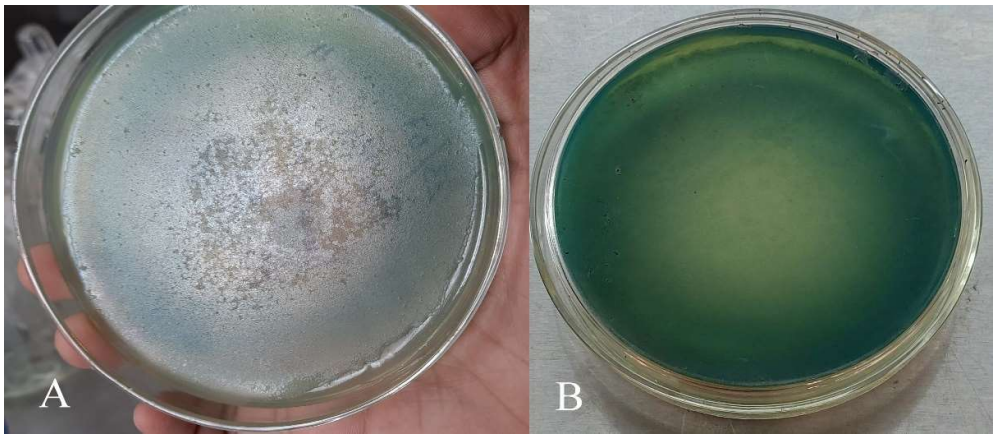

Figure 6: (A) Shows the lyses of *P.aeruginosa* from the inside, whereas (B) shows the clear spot of lyses from outside the plate

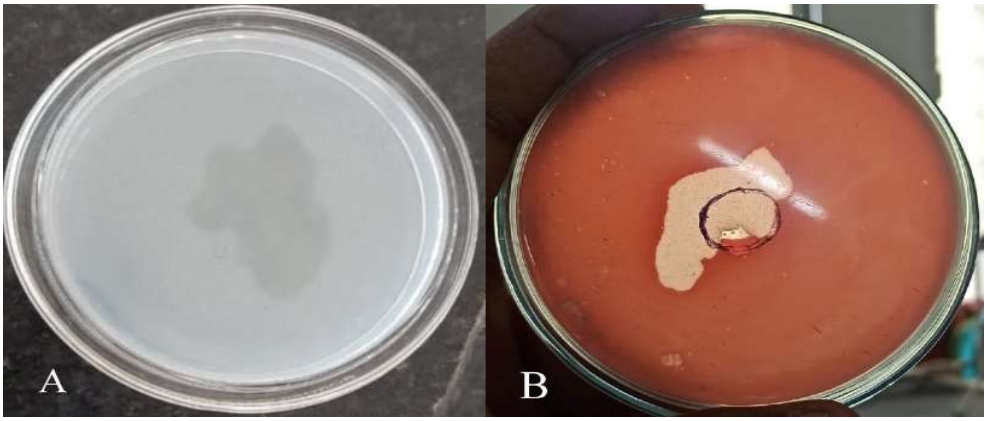

Figure 7: Spot of lytic activity against (A) *E.coli* (B) *S.marcescens*

4. DISCUSSION

In recent years, interest in phages has increased due to their ability to regulate bacterial populations and has spread beyond medical uses and into agriculture, aquaculture, and sewage water treatment. Antimicrobial resistance organisms have increased as a result bacteriophage requirement has also increased. As a result, phage therapy, which employs

bacteriophage as biological control agents, is a viable alternative treatment option for a variety of AMR infections. Raghu et al. have discussed several roles that bacteriophages play in the environment, biofilm control, and water treatment **[17]**. Periasamy and Sundaram have reported the study to remove bacterial pathogens including *E.coli* from hospital wastewater by using bacteriophages. They demonstrated that after 14 hours of incubation, certain E. coli phages can kill the bacterial host **[18]**. Mohammad Mehdi Soltan Dallal and his colleague have reported *E.coli* ATCC (25922) bacteriophage from raw sewage water with a 1mm plague diameter **[19]**.

In comparison to this study, Lytic phages isolated from sewage water against MDR strains, and phages were determined. The double agar layer method is used as primary screening, followed by the spot assay process. The isolated phages have been exposed to different host ranges and have shown a high specific lytic effect by several finding reports early.

5. CONCLUSION

Clear distinct lysis zones formed against host bacteria as a result all phages isolated from sewage are lytic. Following further research, virulent bacteriophages identified against multidrug-resistant strains could be employed as viable medicinal alternatives to antibiotics in humans and animals. This could be due to the increasing use of antibiotics in clinical, veterinary, animal, and agricultural settings, which has caused an increase in antibiotic cure resistance in bacteria. In the future, it is predicted that recovered bacteriophages have the medicinal potential against multi-drug resistant bacteria, which will decrease the use the antibiotics and will also decrease the spreading of superbugs. Furthermore, isolated phages would be used as a bio-remedial factor in the creation of biosensors against food-borne harmful microorganisms.

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