Isolation and Identification of Klebsiella Bacteriophages from Surface Water Samples

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

Department of Pharmacy
Brac University
August 2019

Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.

3. I have acknowledged all main sources of help.

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Approval

The thesis/project titled “Isolation and Identification of Klebsiella Bacteriophages from Surface Water Samples” submitted by Md. Abu Baker Shibly (14346020) of Summer, 2014 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on August 25, 2019.

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Ethics Statement

This project does not involve any kind of animal and human trial.

Abstract

Bacteriophages to the antibiotic resistant bacteria have a broad application in the field of medical science as alternative to the conventional antibiotics. In this research the goal was to isolate and identify the bacteriophages against *Klebsiella pneumoniae* and reference *Klebsiella pneumonia* that are resistant to antibiotics. A series of techniques and protocols were used to get best possible outcome. Several water samples were collected with robust coverage from different ponds and lakes around Bangladesh in order to isolate the bacteriophages. Isolated bacteriophages were applied on the hard agar medium along with soft agar and respective bacterial strains to observe the plaques as bacteriophages. This research may help to reduce the cost, dependency on antibiotics and to kill the bacteria those are resistant to antibiotics as the world is facing a great challenge to control the resistant bacteria from newborn to older individuals.

Keywords: Bacteriophages; antibiotic resistant bacteria; water sample; isolation; control

Dedication

I want to dedicate my project paper to my parents.

Acknowledgement

First of all, all praise to the almighty Allah for whom my thesis have been completed without any major interruption.

Secondly, to my supervisor Dr. Mohd. Raeed Jamiruddin for his kind support and advice in my work. He helped me whenever I needed help.

And finally to my parents and brother, without their support it may not be possible.

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List of Acronyms

DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
PBS	Phosphate Buffered Saline
FDA	Food and Drug Administration
EPA	Environmental Protection Agency
USDA	United States Department of Agriculture
RF	Replicative Form
MDa	Mega Daltons
RPM	Revolutions Per Minute

Chapter 1

Introduction

1.1 Bacteriophage

With the advent of antibiotic resistant bacteria and its spread in the environment, researchers are pivoting back to the old notion of controlling bacteria using bacteriophages (Bragg et al. 2014) . Bacteriophages hold a strong position in the biosphere for their great contribution to medical and non-medical history of the world. Bacteriophages shows their purposes based on their variability, existence and development of viruses by $10^{\wedge 31}$ particles on the earth since three billion years ago (Hatfull and Hendrix 2011). The International Committee for Taxonomy of Viruses demonstrates phage viruses as 61 families, 241 genera that falls bacteriophage in order named Caudovirals having 13 families, 30 genera , capsid protein heads and genetic materials of viruses (Bragg et al. 2014). They can be found in every environment on the earth for example, in surface water commonly in fresh water, sewage water, sea water, soil etc. (Anon n.d.). However, the fundamental use of bacteriophage as medicine was introduced in the treatment of typhoid and bacillary dysentery and more likely in the treatment of infectious diseases caused by bacteria (Sharp 2001). Moreover, antibiotic resistant for example, resistant to penicillin was globally known in 1960s but in recent ampicillin, third generation cephalosporin become resistant to bacteria (Anon 1998). Both in antibiotic resistance and infectious diseases caused by bacteria, bacteriophage kills the bacteria by their lytic and lysogenic pathway (Bragg et al. 2014).

1.2 Historical Background and Research of Bacteriophage

From the beginning of medical evolution, it was considered that cholera, typhoid even fever, infection and other diseases were the prime cause of bacteria for example, *Salmonella typhi* is responsible for typhoid, *Escherichia coli* is responsible for gastroenteritis, *Klebsiella pneumoniae* is responsible for gastrointestinal infection, pneumonia, urinary tract infection etc. taking 1.8 million lives each year in underdeveloped and even in developing countries and more than 76 million American citizens became sick, approximately 3000000 people admitted in hospital and 5000 people died annually (Teaney and Data 2009). Similarly, in the industrial production of food bacteria produce a great obstacle for their pernicious activity (HUDSON et al. 2016) .

However, the therapeutic response of bacteriophages has been claimed independently by Federick d'Harelle in 1917 (Anon 1998). Bacteriophages were successfully applied in curing the *Staplycoccus aureus* skin infection in children in 1919 and in both bubonic patients and treatment of cholera in 1925 (Pelzek et al. 2013a). In 1936, the bacteriophages were introduced in the dairy industries to prevent the contamination along with the test of water quality, different bacteria were selected and bacterial cells were destroyed by 1953 (Sharp 2001). Later in 1960, Lwoff, Jacob, and Monod got their novel prize in 1960 for the confirmation of suppressive action of bacteriophages against the bacterial genetic regulation (Pelzek et al. 2013a).

1.2.1 Modern Research on Bacteriophage

Today bacterial resistance to antibiotic becomes a great alarm (Matsuzaki et al. 2005), bacteriophage therapy is another choice to eradicate this problem though the therapy is being used from very past years but recent scenario pushes the researchers to think about the

bacteriophages in a new angle (Lu and Koeris 2011). Hence, genetically engineered bacteriophages is being produced commercially and used successfully than many other conventional antibiotics (Qadir 2016).

In addition, researchers have isolated bacteriophages from the environmental resources such as water, soil, human and animal feces and a great variety has been found in the phages (Domingo-Calap and Delgado-Martínez 2018). Although bacteriophage therapy is not a modern technique, certain changes have been brought and innovative ideas have been developed in its application (Monk et al. 2010). Current investigation showed that combination of bacterial phage therapy is more effective than single phage therapy (Torres-Barceló et al. 2014). Application of bacteriophage is cheaper than antibiotics because of their availability in the environment and in vitro experiment and these two advantages gives fuels to further research on bacteriophages (Golkar, Bagasra, and Gene Pace 2014). By this sense researchers developed a phage lysine, a new enzymatic antimicrobial agent used to prevent infection caused by gram- positive bacteria, also has the capability to identify the fake strain of the pathogens (Pelzek et al. 2013a). Moreover, different innovative techniques have been discovered to utilize the bacteriophages in the treatment of bacteria causing diseases. Many novel bacteriophages are being identified by research but plating technology is still an effective way for the research on bacteriophages (Keen 2015).

1.2.2 Post Antibiotic Era

Nowadays, as a spontaneous killer of pathogenic bacteria and an alternative way of conventional antibiotics, bacteriophages destroy the disease causing pathogenic bacteria without affecting the micro biome of the human body (Domingo-Calap and Delgado-Martínez 2018). In addition, genetically engineered bacteriophages have a great contribution

to the therapy as adjuvants to the antibiotics administered via different roots and investigations have claimed that the prognosis of the bacteriophage therapy is 82 percent whereas antibiotics have 64 percent recovery (Qadir 2016). Hence, bacteriophages have a great importance to the field of biotechnology because of their unique properties in gene therapy applying as a carriage for the DNA vaccines and preserve the vaccines from deterioration until reaching to the target cells (Haq et al. 2012).Furthermore, bacteriophages have grabbed a large portion of the non-therapeutic field as in the agricultural, food and fermentation industries, by following many companies in different countries are producing phage based product under the surveillance of FDA, EPA, and USDA etc. Beside, various research and survey on bacteriophages are on track for both clinical and non-clinical challenges (Lu and Koeris 2011).

1.3 The Structure of Bacteriophage

By the grace of the invention of electron microscopy the actual structure of the bacteriophages has been revealed successfully. The dimension and morphology of a bacteriophage is identified by electron microscopy. Crick and Watson demonstrated the protein subunit of bacteriophage in 1956 (Leiman et al. 2003).

Different bacteriophages have different conformations. In general the prime component of bacteriophage is nucleic acid containing single or double stranded DNA or RNA covered by a protein capsid called the polygonal head of the bacteriophage. Hence, the prime component of the capsid is capsomeres and the number of capsomere is approximately 2000. The next part of the bacteriophage is collar connected to the tail and the spikes at the base (Haq et al. 2012).

However, there are so many researches have been conducted on bacteriophage structure and morphology, among the phages T bacteriophages studied more times. T bacteriophages include T2, T4 and T6 and interestingly there were no significant differences among three phages (Wurtz 1992).

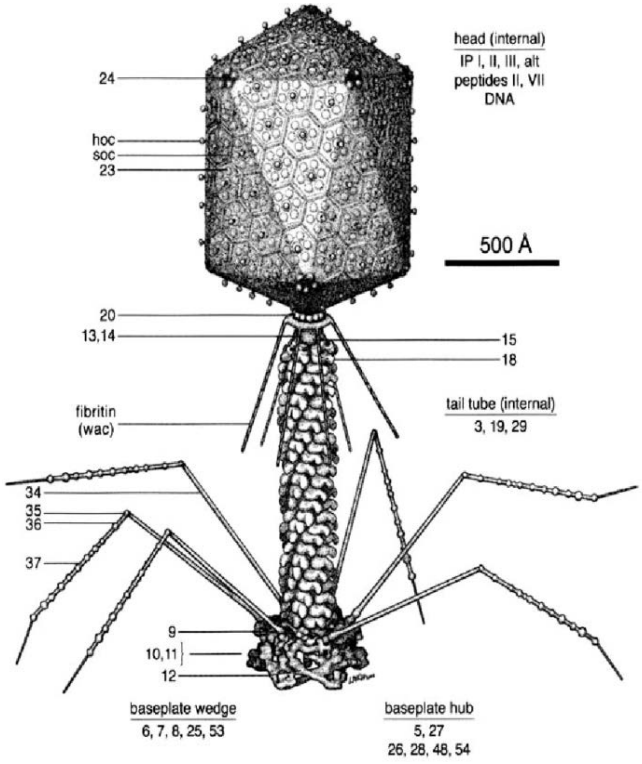

Figure 1 ***:*** *The structure of T4 Bacteriophage and virions are labeled according to gene number (Leiman et al. 2003).*

Head: The structure of the head is considered as icosahedral (Wurtz 1992) and is the combination of over 3000 polypeptide chains of 12 different types of protein and a 172-kbp dsDNA chromosomes. The molecular weight of the head is 194 MDa and molecular weight of the DNA is 112 MDa (Leiman et al. 2003).

Tail: T4 bacteriophage contains a mass or assembly of tail including 22 genes and short and long tail fiber and baseplate. The long tail fiber is considered as a sensor and prime functional

unit of a bacteriophage because *E. coli* bacterium is detected by long tail fiber which is attached to the baseplate (Leiman et al. 2003).

1.4 Classification of Bacteriophage

The classification based on infection or reproduction of bacteriophages is of two types:

a. Lytic or Virulent Bacteriophages: The purpose of the virulent bacteriophages is through the lytic cycle causing the lysis of the host bacterial cell and phages are released into the extracellular space. Example: T-phages (Anon 2012)

b. Temperate Bacteriophages: Temperate bacteriophages works through the replication of DNA into the host cell genome by both lysis and the lysogenic process and a new bacteria is produced along with viral DNA. Example: Lamda phage virus (Anon 2012).

1.4.1 Classification Based on the Conformation or Shape of the Bacteriophages

Table 1: Classification and properties of bacteriophages (Ackermann 2003).

Conformation	Characteristics
a. Tailed Bacteriophages	They are largely distinguished bacterial virus and their tailed structure gives symmetrical conformation and the order is caudovirales (Maniloff, Ackermann, and Jarvis 2004). Their nucleic acid is double stranded DNA (Pelzek et al. 2013a).

b. Polyhedral Bacteriophages	They are DNA contained polyhedral bacteriophage or RNA contained polyhedral bacteriophage of Microviridae family (Maniloff et al. 2004). The nucleic acid is single and double stranded DNA and RNA (Pelzek et al. 2013a).
c. Pleomorphic Bacteriophages	They are double stranded DNA phage virus of Plasmaviridae (Ackermann 2003). Their nucleic acid is double stranded DNA (Pelzek et al. 2013a).
d. Filamentous Bacteriophages	They have filament like shape and becomes double stranded RF DNA after infecting the bacteria of Inoviride family (Ackermann 2003) and the nucleic acid is single and double stranded DNA (Pelzek et al. 2013a).

1.5 The life cycle of bacteriophages

The research shows that the life of the bacteriophage is unpredictable (Hatfull and Hendrix 2011) because of their diversity, ecology and coevolution with bacteria (Domingo-Calap and Delgado-Martínez 2018). The life cycle of bacteriophage is depended on some factors including the count of genetic materials they secret into the host bacterial cell, the time from the causing of infection of bacteria to the secretion of genetic materials and the rate of adsorption or the attachment period of the bacteriophage on to the host bacterial cell (Keen

2014).However, there are two developmental cycle, one is lytic and another is lysogenic cycle by which the life cycle of a bacteriophage is conducted (Pelzek et al. 2013a).

Lytic Cycle: In the lytic cycle of bacteriophage an order of stages are involved by which the host bacterial cell is lysed (Mathur, Vidhani, and Mehndiratta 2003). First of all, the bacteriophage comes in contact with the surface of the bacterial host cell (Karthik et al. 2014). More the attachment time or adsorption rate more the capability of causing infection (Keen 2014). Secondly, the bacteriophage penetrates the host cell and secretes the phage genetic materials or daughter particles into the host bacterial cell (Shao and Wang 2008). Finally, the lysis of host bacterial cell occurs during the finishing stage of the replication cycle causing the rupture of the cell and the phages become free from the host bacterial cell (Karthik et al. 2014).

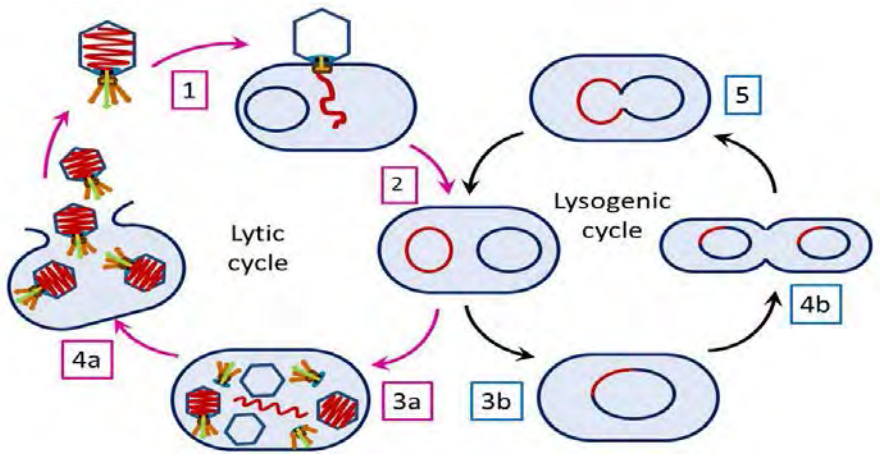

Figure 2: Two life cycles of the bacteriophage. 1- Adsorption of host cell; DNA in the lytic and lysogenic cycle; 3a- new DNA is produced; 4a- lysis of the cell; 3b and 4b- representing the lysogenic cycle of the phage; 5- beginning of lytic cycle (Anon 2012).

Lysogenic Cycle: In the lysogenic cycle, the genome of the bacteriophage is insisted in the host bacterial cell without causing major metabolic impact to the cell (Mathur et al. 2003).

The DNA of the phage is attached to the specific part of the bacterial chromosome and brought phenotypic changes to the cell (Pelzek et al. 2013a). The phage DNA is isolated as a different part along the host cell and further initiates the lytic cycle (Karthik et al. 2014) as a hidden scheme of infection at quiet recurrence (Mathur et al. 2003).

1.6 Seasonal Fluctuation of Bacteriophages

Bacteriophages are enormous in number and existed in every environment for example, water, soil, sand and subsequent part of the earth (Sharp 2001). The contribution of bacteriophages takes a strong position in the field of ecology by keeping the balance among the climate, weather and the life threating global warming to protect the existence of lives (Keen 2015). However, the seasonal fluctuation has both positive and negative effects on bacteriophages by bringing changes in their nature (Maurice et al. 2010). Environmental condition changes in different seasons causing the variations in the concentration of bacteriophages as more phages are found in spring, summer and autumn but less in winter (Drucker and Dutova 2009) . Likewise, heavy rainfall in summer increases the amount of surface water causing dilution of colony of bacteriophages and can spread widely in water (Borsherim Knut 1993). Moreover, the toxic materials that are lethal to the bacteriophages are lessen in concentration in summer (Maurice et al. 2010) although significant amount of bacteriophages have been found in polluted seawater (Berry and Noton 1976). In addition, higher temperature affects the survival and activity of bacteriophages (Jończyk et al. 2011), similarly iceberg indirectly affect the infectability of bacteriophages causing less sensitive to bacteria as iceberg injures the bacterial cell (Maurice et al. 2010).

1.7 Isolation and Culture of Klebsiella Bacteriophage

Bacteriophages have a wide use in both of industry and medical area because of their good and immediate response, advantages and as an alternative way of application to a newly appearing bacteria (Colindres, Leon, and Holganza 2011). Based on the availability, diversity and capability of causing diseases varies different types bacteria found in the environment. Amongst them gram negative *Klebsiella pneumoniae* and Reference *Klebsiella pneumoniae* (Bassetti et al. 2018) are quite available in nature and causes strong infectious disease in human body preferably nosocomial, skin, respiratory tract infection (Struve, Bojer, and Krogfelt 2008) and becomes resistant to antibiotics by making wide- spectrum beta-lactamase (Kumari, Harjai, and Chhibber 2010). However, the bacteriophages collected from water sample(Titer 2016) are highly specific against the diseases caused by the klebsiella bacteria (Anon n.d.).

Collection of Samples: The samples of the bacteriophages are collected as surface water and stored at 4 degree Celsius temperature before using for the next step (Colindres et al. 2011). In our research, we collected ten water samples from different lakes and ponds around Bangladesh. The samples were tested to isolate bacteriophages amongst the three were from the Jamuna, Padma and Buriganga River and rest others were from different lakes and ponds.

Bacterial Strains: In this research, we used two types of bacterial strains *Klebsiella pneumoniae* and Reference *Klebsiella pneumonia* for the isolation of bacteriophages from the collected samples.

Chapter 2

Components

2.1 Reagents and Tools

The strains of the respective bacteria for culture and isolation of bacteriophage

Cultural medium: 1. Nutrient broth 2. Nutrient agar

Filtered bacteriophage from reserve

Phosphate Buffer Saline

Duran 250 ml bottles (sterilized): Used for sample collection

0.22 μm size syringe filters

Several numbers of sterile beakers in different sizes

Several numbers of sterile vials

Sterile clean cotton

Ethanol (70%) as disinfectant

90 mm sterile plates

Several numbers of sterilized test tubes

Different sizes conical flask

Foil paper of aluminum

Whatman filter paper

2.2 Apparatus (Machines)

Incubator for bacterial growth

Refrigerator used to reserve and store the samples, cultured plates and reagents

Laminar air flow

Incubator with shaker

Centrifugation machine

Autoclave machine as steam sterilizer

Hot water bath for soft agar

Chapter 3

Methodology

3.1 Streaking and Preparation of Broth-Bacterial Suspension in Test Tube

i. First of all, several sterile plates are made ready for the pure culture of the bacteria on medium.

ii. Then 2.8% w/v nutrient agar is prepared in the flask and autoclaved it at 121°C for 20 minutes.

iii. After autoclaving agar is poured in to the plates when the temperature is down to 55°C and let to solidify.

iv. After that strains of *Klebsiella pneumoniae and* reference *Klebsiella pneumoniae* are streaked on the agar mediums and kept into the incubator for 24 hours at 37°C for the growth.

v. Several test tubes are prepared with nutrient broth (0.7 gm/40 ml) and respective strains of the bacteria that cultured previously are added to the test tubes and kept in the incubator for 24 hours at 37°C for the preparation of broth-bacterial suspension.

3.2 Filtration of Collected Samples

i. At first the centrifugation of collected sample is done for 10 minutes with the rotation of 10000 rpm.

ii. After that the sample is filtered for several times with Whatman filter paper to remove the impurities.

3.3 Preparation of Bacteriophage for Dilution

i. At first 0.9 ml broth-bacterial suspension of *Klebsiella pneumoniae* and reference *Klebsiella pneumoniae* are poured in different small beakers and then 0.3 ml previously filtered sample is mixed and kept the beakers in incubator at 37°C for 10 minutes.

ii. After incubation, the mixture is filtered with micro filter to remove the bacteria.

3.4 Preparation of Phage Dilution

i. Firstly, 0.9 ml PBS is placed in a beaker and 0.1 ml previously prepared bacteriophage is mixed with it.

ii. Then the mixture is diluted to 10^{-2}, 10^{-4}, 10^{-6} and 10^{-8} concentrations with PBS in small vials. The dilution is done for *Klebaiella pneumonia* and reference *Klebsiella pneumoniae* and concentration is noted on the surface of the vials.

3.5 Preparation of Diluted Phage Containing Soft Agar

i. First of all, 0.1 ml broth-bacterial suspension of *Klebsiella pneuminiae and* reference *Klebsiella pneumoniae* is taken in different test tubes and 0.1 ml of every diluted bacteriophage is added to the test tubes.

ii. Then the test tubes are kept in the shaking incubator at 37°C with the rotation of 80 rpm for 10 minutes.

iii. After incubation 3 ml soft agar (0.7% w/v) is added to the test tubes.

iv. Then 10 µl of every dilution of phage is place in the different sectors on the hard agar plates (1.8% w/v) and incubation for 24 hours at 37°C.

3.6 Selection of Dilution and Confirmation

i. The different sectors of 2 plates of *Klebsiella pneumoniae* and reference *Klebsiella pneumoniae* with different dilutions are observed for the confirmation of the plaques as respective concentration.

ii. After the confirmation of the concentration test tubes are prepared according to previous methods.

iii. Finally, the bacteriophages are applied against respective bacteria on hard agar medium by following:

1. *Klebsiella pneumoniae* phage vs. *Klebsiella pneumoniae*

2. Reference *Klebsiella pneumoniae* phage vs. reference *Klebsiella pneumoniae*

Chapter 4

Results

4.1 Sample 1: Buriganga River

Date of Collection: 25.05.2019

Location: Geographically the position of the collected sample source as latitude is 23°37′59.99″ N and 90°25′59.99″ E as longitude.

Figure 3 ***:*** *Buriganga River (BR)*

*Figure 4****:*** *Bacteriophage from Buriganga River (BR)*

Observation: Some traces of plaques have been found with this sample but requires confirmation.

4.2 Sample 2: KYAMCH Pond (Sirajganj)

Date of Collection: 13.07.2019

Location: Geographically the position of the collected sample source as latitude is 24°19′10.9″ N and 89°41′51.7″ E as longitude.

Figure 5: KYAMCH Pond

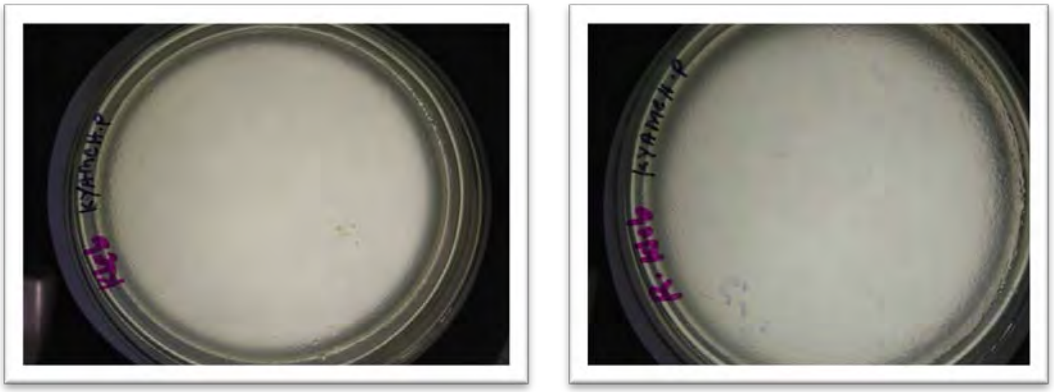

Figure 6: Bacteriophage from KYAMCH Pond

Observation: No clear plaques have been found with this sample.

4.3 Sample 3: Hospital Para Pond (Sirajgang)

Date of Collection: 19.06.2019

Location: Geographically the position of the collected sample source as latitude is 24°19′17.5″ N and 89°41′14.1″ E as longitude.

Figure 7: Hospital Para Pond

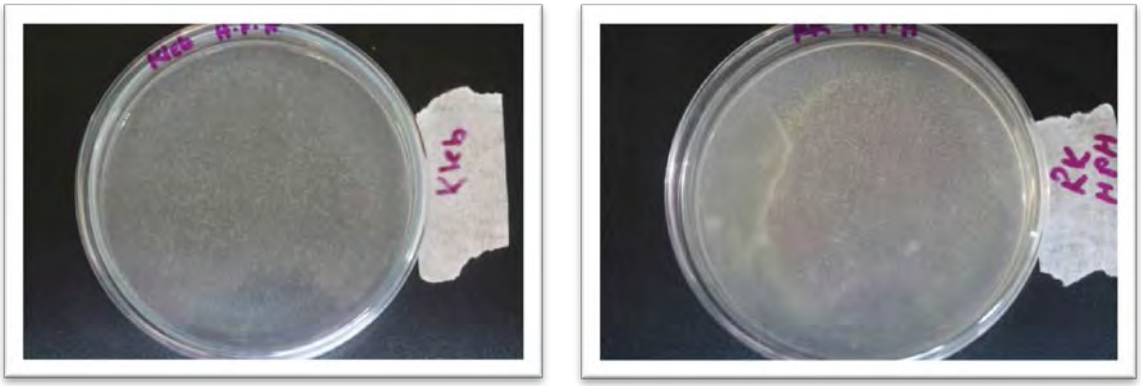

Figure 8: Bacteriophage from Hospital Para Pond

Observation: No clear plaques have been observed with this sample.

4.4 Sample 4: Nanua Dighi (Comilla)

Date of Collection: 06.07.2019

Location: Geographically the position of the collected sample source as latitude is 23°45′91.74″ N and 91°18′90.40 E as longitude.

Figure 9: Nanua Dighi

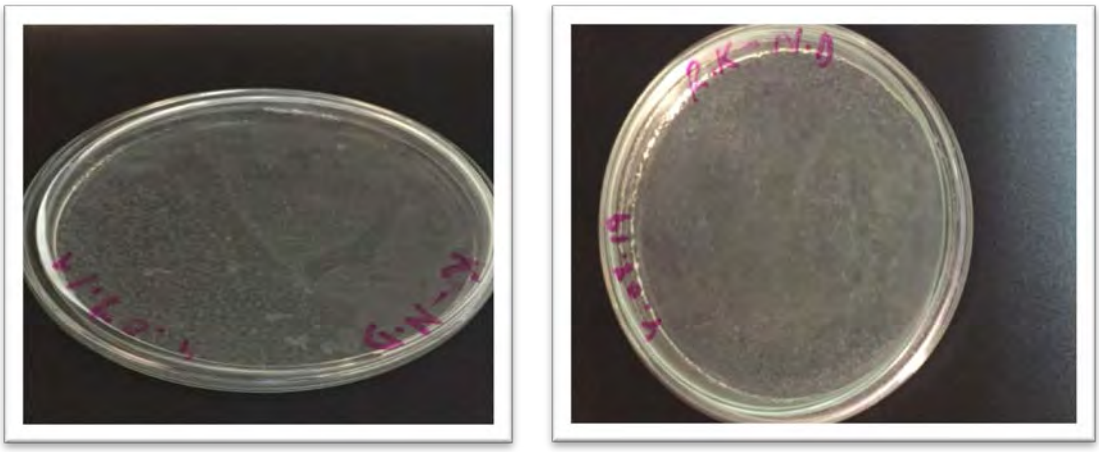

Figure 10: Bacteriophage from Nanua Dighi

Observation: No clear plaques have been found with this sample.

4.5 Sample 5: Rani Dighi (Comilla)

Date of Collection: 06.07.2019

Location: Geographically the position of the collected sample source as latitude is 23°45′93.42″ N and 91°18′30.57 E as longitude.

Figure 11: Ranir Dighi (RD)

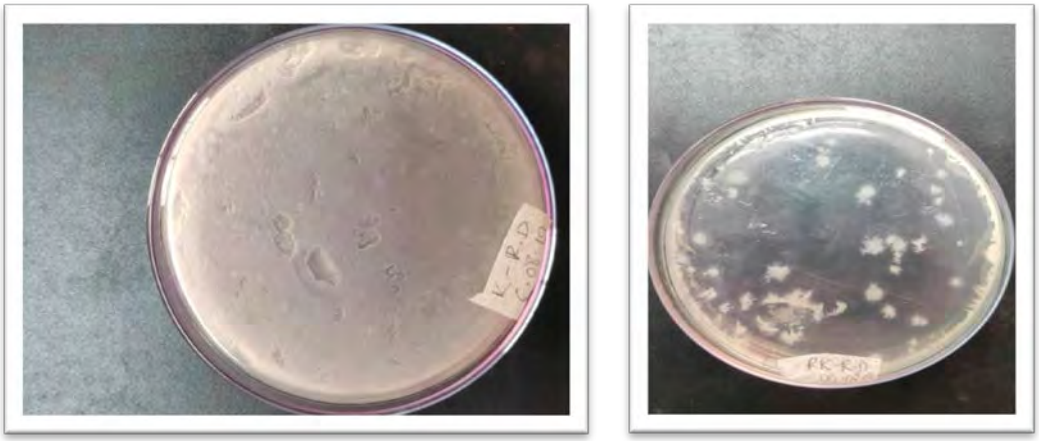

Figure 12: Bacteriophage from Ranir Dighi (RD)

Observation: No clear plaques have been observed with the sample.

4.6 Sample 6: Jubilee Tank (Faridpur)

Date of Collection: 15.05.2019

Location: Geographically the position of the collected sample source as latitude is 23.56023° N and 89.8381° E as longitude.

Figure 13: Jubilee Tank

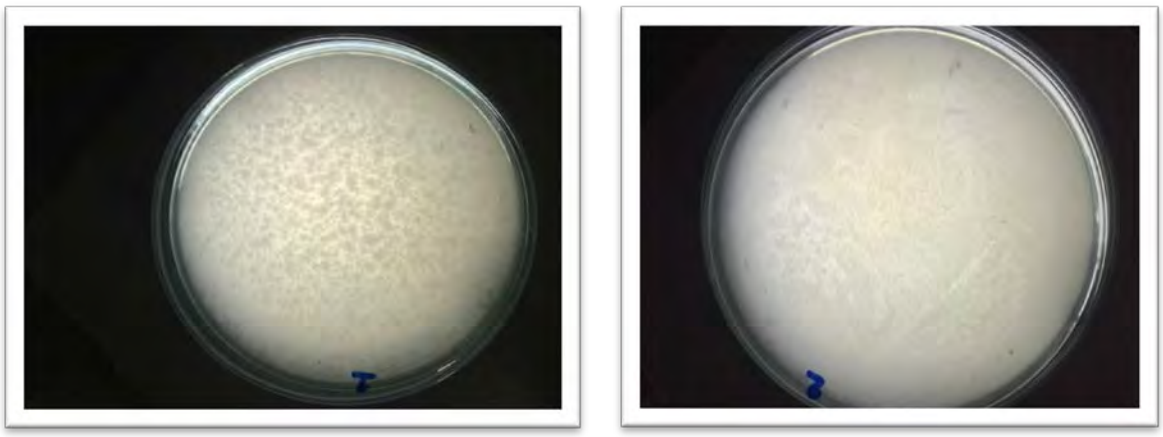

Figure 14: Bacteriophage from Jubilee Tank (JT)

Observation: No clear plaques have been observed with this water sample.

4.7 Results of Samples without Dilution for *Bacillus subtilis*

4.7.1 Sample 7: icddr, b Pond (Mohakhali)

Date of Collection: 10.03.2019

Location: Geographically the position of the collected sample source as latitude is 23.776910° N and 90.401712° E as longitude.

*Figure 15**:** Icddr,b pond*

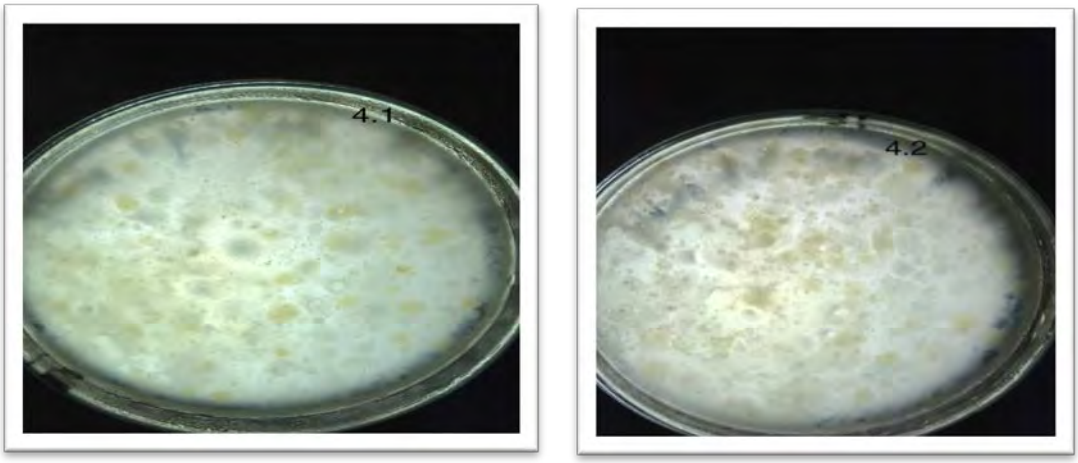

*Figure 16**:** Bacteriophage from iccddr,b Pond*

Observation: No clear plaques have been observed with this sample.

4.7.2 Sample 8: Arambagh Pond

Date of Collection: 22.02.2019

Location: Geographically the position of the collected sample source as latitude is 23.587595° N and 89.857616° E as longitude.

Figure 17: Arambagh Pond

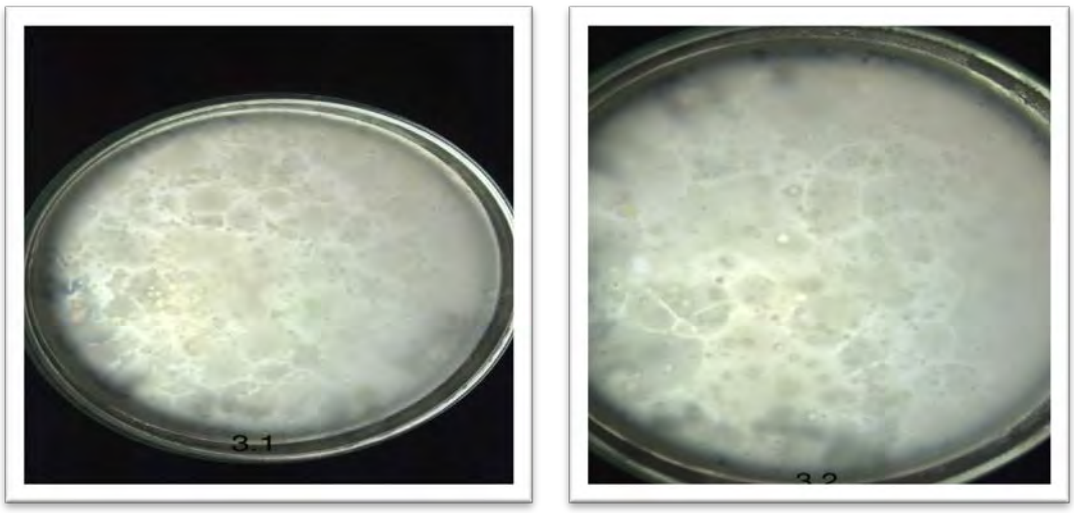

Figure 18: Bacteriophage from Arambagh Pond

Observation: No clear plaques have been found with the sample.

4.7.3 Sample 9: Narshindi

Date of Collection: 10.03.2019

Location: Geographically the position of the collected sample source as latitude is 23.776049° N and 90.397193° E as longitude.

Figure 19: Narshindi Pond

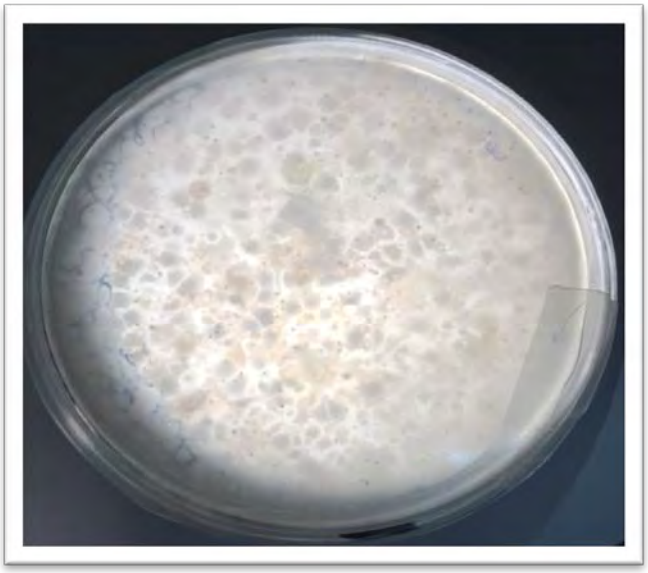

Figure 20: Bacteriophage from the pond in Narshindi

Observation: No clear plaques have been observed the sample.

4.7.4 Sample 10: Padma River

Date of Collection: 10.02.2019

Location: Geographically the position of the collected sample source as latitude is 23.4662° N and 90.2897° E as longitude.

Figure 21: Padma River

Figure 22: Bacteriophage from Padma River

Observation: No clear plaques have been observed with this collected water sample.

Chapter 5

Discussion

Ten samples were collected from different ponds and lakes around Bangladesh because infectability and surface water sources contain different types and amount of bacteriophages. Usually the plaques are formed as clear zone in the plates representing the bacteriophages against respective bacteria. In this research, only one sample collected from Buriganga River showed some traces of plaques but requires confirmation. However, we followed processes used in researches in different countries, there might have reasons for negative results. As a reason we collected 250 ml of each sample in Duran bottle was very small in amount whereas we should collect large amount of samples compared to the sources. Each of the collected samples requires to be tested thrice in times more for the confirmation of the plaques where we were able to perform only one test. In addition, the amount of bacteriophages varies in different seasons as in winter, spring, summer and fall can be a reason for the negative results. Furthermore, unavailability of schedule of machines, contaminants in nutrient agar and broth and cross contamination as other project members worked in same lab at same time might be another reason. Moreover, we use different techniques and protocols to find out the bacteriophages were reliable but lengthy, requires more time for the confirmation and isolation of bacteriophages. Hence, if we revise the process, control the contamination and ensure the proper amount of time and schedule, positive results can be expected as the plaques have been found in researches in India and other countries.

Chapter 6

Conclusion

Our prime goal in this project is to isolate and identify the bacteriophages while antibiotic resistant bacteria becoming strong gradually and insensitive to conventional antibiotics. We collected several samples from surface water around Bangladesh and used different techniques and protocols to isolate and identify the bacteriophages at different concentrations. The bacteriophages will infect and kill the bacterial strains that are resistant to the antibiotics and further will reduce cost and dependency on antibiotics. So, researchers should emphasize on such project to save the lives, to save the world.

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