Isolation of Bacteriophage Using E. Coli ATCC-25922 And E3 Strain from Water Samples Around Bangladesh

By
Rayhana Alam
15146009

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

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Declaration

It is hereby declared that

1. The thesis submitted is my/our 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.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

Student's Full Name & Signature:

Rayhana Alam
15146009

Approval

The thesis/project titled "Isolation of Bacteriophage Using E. Coli ATCC-25922 And E3 Strains from Water Samples Around Bangladesh" submitted by Rayhana Alam (15146009) of Spring 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on 22nd August, 2019.

Examining Committee:

Supervisor:
(Member)

Dr. Mohd. Raeed Jamiruddin, PhD
Assistant Professor, Department of pharmacy
Brac University

Program Coordinator:
(Member)

Hasina Yasmin, PhD
Associate Professor, Department of Pharmacy
Brac University

Departmental Head:
(Chair)

Eva Rahman Kabir, PhD
Professor and Chairperson, Department of Pharmacy
Brac University

Ethics Statement

This study does not contain any human or animal trail or does not have any unethical occurrence.

Abstract

Bacteriophages are the most abundant organism on the Earth having simple structure but diverse characteristics. Since their discovery in 1915 till now they have proved to be useful to human as phage therapy, diagnostic weapon, genetic screening tool, detector of pathogenic bacteria, therapeutic agent and so on. With the recent increase of antibiotic resistance, phage research has attracted interest because of their ability to infect and kill bacteria without causing any harm to human. This experiment is based on the phage collection or isolation from environmental samples around Bangladesh to find out any presence of phages using two types of bacterial strains where one is the standard of the other. It contains protocols that have been tried in the laboratory to see the presence of phages and the outcomes from the experiments. The aim of this project is to detect any presence of phage in environment samples of Bangladesh.

Keywords: Bacteriophage; phage; phage therapy; bacterial strain; phage isolation.

Dedication

This project named titled “Isolation of Bacteriophage Using E. Coli ATCC-25922 and E3 Strains from Water Samples Around Bangladesh” is dedicated to Department of Pharmacy, Brac University.

Also I would like to dedicate this work to my family, all my teachers who have supported me throughout my bachelor of Pharmacy (Hons) period and my friends for being by my side.

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ATCC	American Type Culture Collection
E. coli	*Escherichia coli*

Chapter:1

Introduction

1.1. Background of Virus and Bacteriophage Discovery:

The germ theory of diseases confirmed the fact that microorganisms was the causative agents for many diseases. (Garcia, 2018) However, for certain diseases the causative agents could not be isolated. Though vaccines were developed for these uncultured agents, they still posed an enigma to the scientists who were not able to isolate them using normal microbiological culturing techniques (Plotkin, 2014).

It was towards the end of 18th century, Dmitri Iwanovsky was able to observe that irrespective of using a filter he was not able to isolate the causative agent of the tobacco mosaic disease. (Britannica, 2019 updated) (The Discovery of Viruses). However, it was almost half a decade later while carrying out the same experiment, Martinus Beijerinck, was able to observe that fluid thus filtered was able to retain the infectivity. He termed it Virus, which in Latin means poison. (The Discovery of Viruses) (A, 1999). Later on, various diseases were shown to contain such infectivity in the filtered fluid.

In 1930, with the invention of both electron microscope and fine filters, it was possible to see the viruses for the first time, thus establishing the fact that it was viruses not bacteria that was the cause of certain infection. (The Discovery of Viruses)

In 1915, Fredrick Twort while studying vaccines, found some white spotted area on his plates that were seemed to be some waned bacteria upon closer look but could not give proper cause behind this incident. (Keen, 2015). Later in 1917, Felix d'Herelle got the same result and were convinced it to be a type of virus and named them bacteriophage. (Keen, 2015)(Criscuolo, Spadini, Lamanna, Ferro, & Burioni, 2017).

1.2. Bacteriophage:

Bacteriophage the name signifies bacteria killing agents, are the most abundant and simple microorganisms that live upon infecting bacteria (Criscuolo et al., 2017)(Haq, Chaudhry, Akhtar, Andleeb, & Qadri, 2012). Almost 10^31 phages in the ecological community which is equivalent to 10times the amount of bacteria, ensures their presence where they find their bacterial host and the abundancy may vary according to location or seasons like that the amount of phages was abundant in coastal water, oceans or in greater amount in lakes.(Keen, 2015) (Mohamed Elbreki, 26 March 2014) (Mohamed Elbreki, 26 March 2014). This presence of phages differs due to seasonal variation and location (Chibani-chennoufi, Bruttin, Brüssow, Dillmann, & Bru, 2004)(Keen, 2015) (agata Jurczak-Kurek, 04 October 2016).While surrounded by bunch of bacteria, phages infect only the specific ones others remaining unaffected. When the bacteriophage finds its specific bacteria it attaches itself with the host and reproduce only in presence of bacterial host cell. They are harmless to human. (Kasman & Porter., january 2019)

1.3. Structure of Bacteriophage:

Phages consist of a core genetic material called nucleic acid, surrounded by a protein capsid and the nucleic acid either contains DNA or RNA and maybe double or single stranded respectively. This part is known as the head. The head is extended having six sides polygon and surrounded by almost 2000 capsid. (Keen, 2015)(Haq et al., 2012)

Below head they have a collar and the collar is extended to tail and six spikes and tail fiber which is on the baseplate. The tail is tubular structure and the baseplate is hexagonal. (Haq et al., 2012).

There are three main structural forms of a phage like an icosahedral (20-sided) head with a tail, an icosahedral head without a tail, and a filamentous form. (Criscuolo et al., 2017)

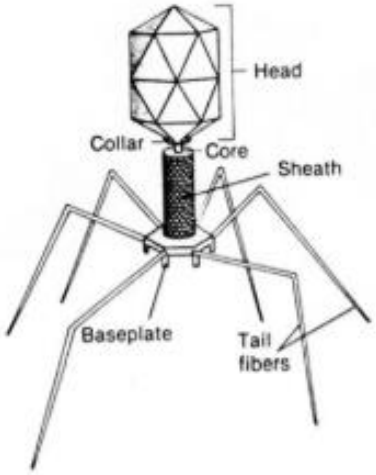

Figure 1: Model of T4 bacteriophage (Kenneth Todar, 2008-2012)

1.4. Life Cycle of Bacteriophage:

Phages have two distinct life cycle, namely lytic and lysogenic life cycle.

LYTIC CYCLE: In lytic cycle bacteria dies through viral infection which occurs due to the insertion of viral genome inside the bacterial cell. (Criscuolo et al., 2017)(Haq et al., 2012)

STAGES OF LYTIC CYCLE:

Attachment: Firstly phage is attached to the surface of the specific host bacteria with the help of the tips of the tail fiber and the process of attachment is called adsorption. (Kasman & Porter., january 2019) (Bacteriophages: Structure and Reproduction (Replication Cycle))

Entry or penetration: The phage penetrates bacterial cell wall, inserts its genome into the cytoplasm of the bacterium which is called penetration. (Bacteriophages: Structure and Reproduction (Replication Cycle))

DNA replication and protein synthesis: Immediately after penetration the phage DNA synthesizes which later break down the bacterial DNA and others are used to replicate DNA. The synthesized DNA later produces protein. (Bacteriophages: Structure and Reproduction (Replication Cycle))

Assembly of new phage or virion: The capsid protein and the new DNA together form new phage particle and those are assembled. (Bacteriophages: Structure and Reproduction (Replication Cycle))

Lysis: The cell swells and burst causing cell lysis and the new phages come out and ready for further infection. (Bacteriophages: Structure and Reproduction (Replication Cycle))

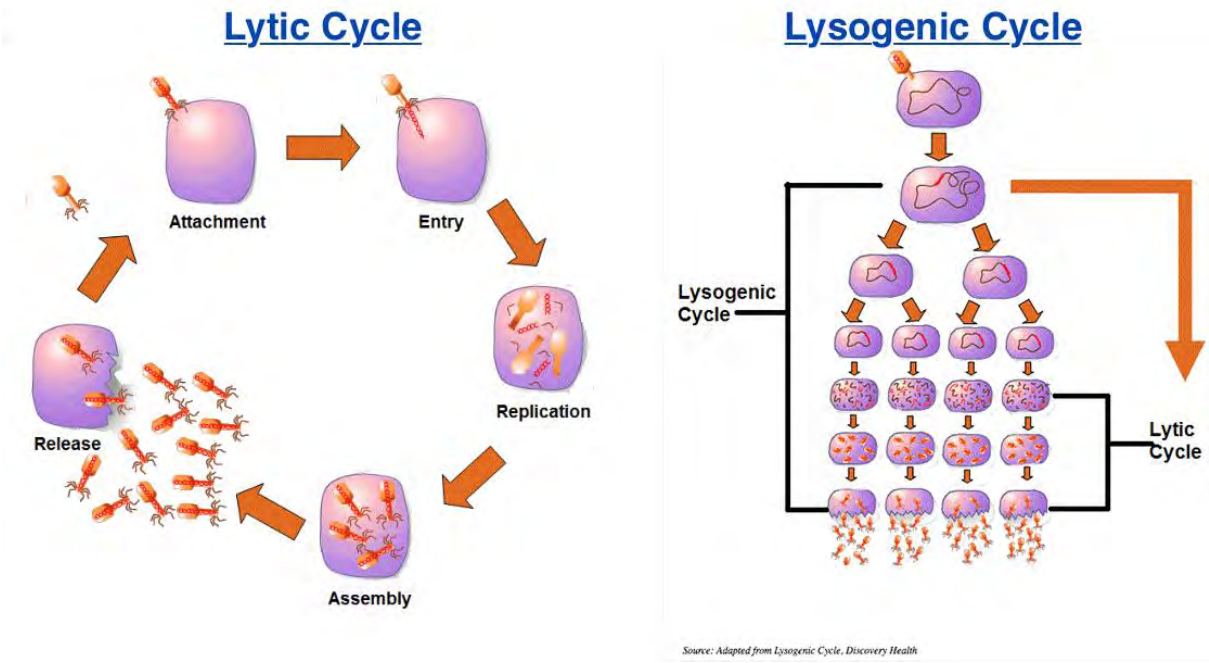

Figure 2: Lytic and lysogenic cycle (adapted from Discovery health)

LYSOGENIC CYCLE: Inside the bacterial cell, phage inserting its genome does not kill the host rather both of them reproduce themselves containing viral genome inside the host daughter cell.(Haq et al., 2012)(Criscuolo et al., 2017). Upon UV-exposure and other environmental condition, phages can undergo lytic cycle killing their host.. (Kasman & Porter., january 2019) (Bacteriophages: Structure and Reproduction (Replication Cycle))

1.5. Types of Bacteriophage:

Depending on the style of reproduction, phages can be of two types. Such as Virulent and Temperate phages:

Virulent bacteriophages: Phages which reproduce through lytic cycle like T-phages are virulent bacteriophage.(Pelzek, Schuch, Schmitz, & Fischetti, 2013)

Temperate bacteriophages: Phages like lambda virus are temperate virus which reproduce using both cycles.(Pelzek et al., 2013)

Table 1. Depending on the presence of double or single stranded DNA or RNA bacteriophage can be classified into the following:

Family of bacteriophage	Double or single stranded DNA or RNA	examples
Myoviridae	Double stranded DNA	Coliphage T2, T4, T6
Styloviridae	Double stranded DNA	Coliphage T1, T5
Pedoviridae	Double stranded DNA	Coliphage T3, T7
Corticoviridae	Double stranded DNA	*Pseudomonas* phage MP2
Tectiviridae	Double stranded DNA	Phage PRD 1
Plasmaviridae	Double stranded DNA	Phage MV- L2
Microviridae	Single stranded DNA	*E. coli* phage φX174

Inoviridae	Single stranded DNA	*E.coli* phage fd
Cystoviridae	Double stranded RNA	Phage φ6
Leviviridae (Classification of Bacteriophage | Bacterial Virus)	Single stranded RNA	Phage MS2

1.6. Significance of Bacteriophage:

Bacteriophage helps in the identification and detection of pathogenic bacteria through encoding of pathogenic toxin. For example: diphtheria toxin of *Corynebacterium diphtheriae* is encoded with the phage. (Kasman & Porter., january 2019)

Bacteriophage do not harm human rather each of them only infect a special species of bacterial strain. (Kasman & Porter., january 2019)

Phage is also used in genetic engineering as a medium to transfer genetic material. (Kasman & Porter., january 2019)

In case of any environmental samples, it can work as a good sensor to spot the presence of the specific host and serves as a microbiological detector. (Kasman & Porter., january 2019)

It can be used in "phage therapy" to invade many deadly bacterial infection or disease. (Moelling, Broecker, & Willy, 2018)

They help in the processing of organic materials that are harmful for the ecological community. (Chibani-chennoufi et al., 2004)

1.7. Bacteriophage Collection and Isolation:

Collection of sample: Environment is a vast source of bacteriophage and phages can be collected from any sample where there is bacterial growth. (Mohamed Elbreki, 26 March 2014)(Keen, 2015). For collection of sample water bodies like rivers and lakes around Bangladesh were choosen since bacteriophage were successfully isolated from rivers and lakes in early researches in Bangladesh (Naser et al., 2017) (Shah M. Faruque N. C., 2003) (Shah M. Faruque I. B., 2005). However, phage isolation in Poland(Jo & Zaczek, 2016), Ganga and Jamuna rivers of India(Terms, 2016)(HUDSON, BILLINGTON, CAREY-SMITH, & GREENING, 2016), few environmental sources of Wuhan, Hubei-China(Huang et al., 2018), also a great concentration of phages found in lakes by Norwegian group (Chibani-chennoufi et al., 2004) and many others were the encouragement behind the water sample collection from around Bangladesh. A total of 10 water samples were tested to isolate phages of which three are Jamuna, Padma and Buriganga rivers of Bangladesh and the rest seven were lakes and ponds. The samples were collected in sterile Duran bottles.

Bacterial strain: Three types of bacterial strain *Escherichia coli* or E. coli strain, E. coli type E3 and E. coli ATCC 25922 were used in the isolation of phages from the samples. E. coli is a gram negative in nature.

ATCC is the American Type Culture Collection organization for collecting, storing and distributing standard reference microorganism. E. coli ATCC is an antimicrobial susceptibility testing strain which was used as reference strain against the other two E. coli strains.

Chapter:2.

Methodology:

2.1. Bacterial Broth Culture Preparation

i. Nutrient broth was prepared having approximately 0.7-0.8% concentration.

ii. For each test tube 10ml liquid broth were prepared with 10ml distilled water and 0.175gm nutrient broth.

iii. Then to the test tube of 10ml nutrient broth, bacterial strains from the cultured Petri dish from previously cultured plates were taken with the help of a loop and carefully added and stirred.

iv. The test tubes with the bacterial strains were kept for 24hours at 37 degrees Celsius at the incubator to let the bacteria grow.

2.2. Preparation of Liquid Phage Culture:

i. From the cultured broth from 2.1 bacterial broth is taken and mixed with isolated phage in a ratio 3:1

ii. The liquid culture was prepared for E. coli ATCC and E3 bacterial strains separately in two separate test tubes.

iii. The prepared test tubes were kept at 37 degrees in shaking incubator for 20 minutes.

iv. After 20minutes, the test tubes were taken out and filtered to ensure absence of any bacteria or particles.

2.3. Preparation Top and Bottom Agar Layers:

i. Nutrient agar was layer prepared for bottom and another agar layer was prepared separately and autoclaved at 121 degrees Celsius.

ii. The agar was kept to cool down at 55-65 degrees Celsius.

iii. Then the bottom layer was poured at the bottom for a solid support on the petri dish and allowed to solidify. This was done twice for E3 and E. coli ATCC bacterial strains.

iv. When solidified, the above agar layer with diluted phage solution was poured on it shown in 2.5. When ready, the plates were kept at 37 degrees Celsius in incubator.

2.4. Preparation of Phage Titration:

i. The phage was then diluted to different concentrations with a buffer solution in small vials. The tittering was done for E3 and E. coli ATCC. The concentrations were marked on the vials to avoid any confusion.

2.5. Preparation of Diluted Phage Containing Agar for Overlay Tittering:

i. In a test tube cultured bacteria and selected dilution containing phage sample are mixed and incubated at 70-80rpm.

ii. This was done for ATCC E. coli and E3 bacterial strains separately.

iii. After 15minutes of shaking and incubation, 10 µl from each dilution were spotted on the agar plate after mixing with 3ml soft agar leaving some area in between and each sector of the plate was marked according to spot of diluted concentration.

iv. The plate was now kept for incubation for 18-24hours.

v. The plates are now ready for results.

Chapter 3: Results

3.1. Sample 1

3.1.1. Place of collection:

The sample was collected from River Buriganga having Latitude 23°37’59.99” N and Longitude 90°25’59.99” E. Out of total 10 samples, 2 were collected from rivers and Buriganga being one of them because river is termed to be a good source of phages.

Figure 3: Name of place: River Buriganga

3.1.2. Outcome of E3: Selected Dilution concentration was 10^ -6 for this sample.

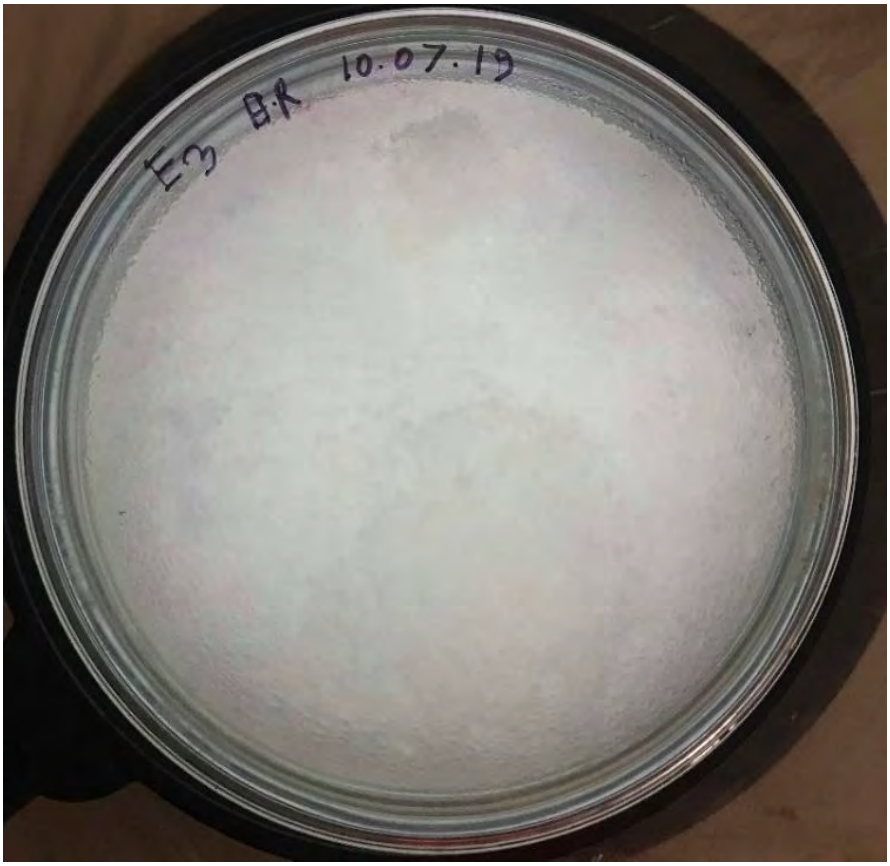

Figure 4 : Plate of E3 from sample Buriganga

Results: the plate is clear and no zone of inhibition is seen above, which means there are no E3 phage which was present in the sample collected when tested once. Therefore, the result is negative.

Outcome of E. coli ATCC: Selected Dilution concentration was 10^ -6 for this sample.

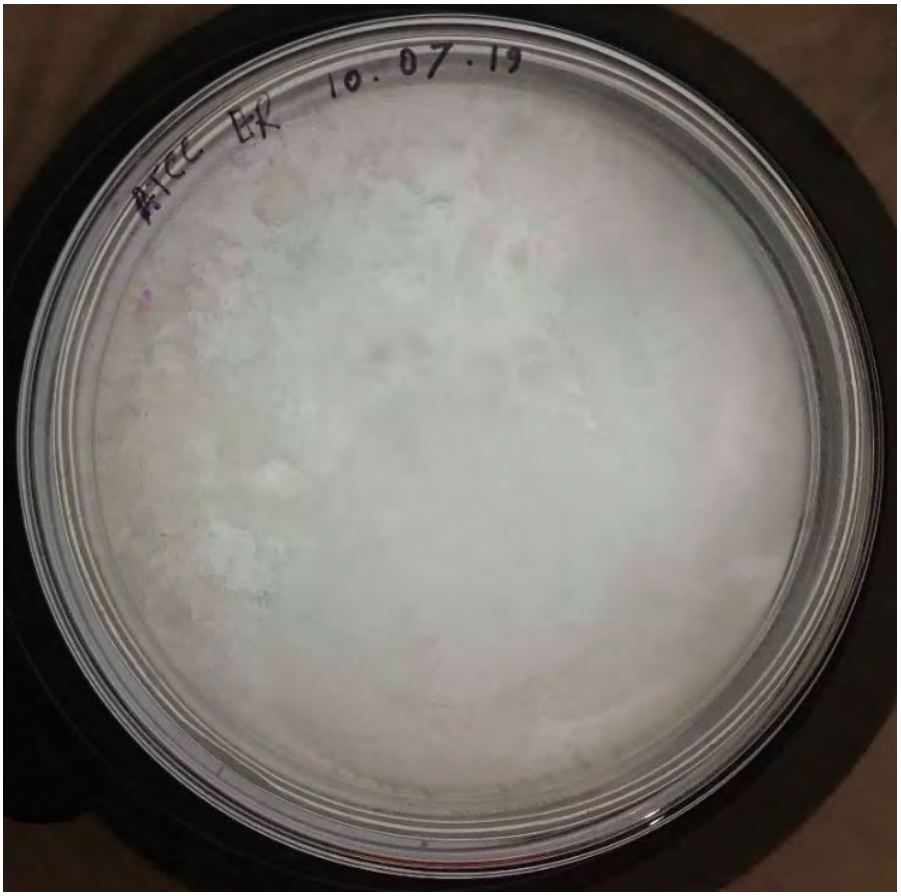

Figure 5: Plate of E. coli ATCC from sample Buriganga

Results: the above plate is the plate of E. coli ATCC done from river Buriganga and done once. The plate does not contain any zone of inhibition, which means there are no coliphage present in the sample. Therefore, the result is negative.

3.2. Sample 2

3.2.1. Place of collection:

The pond Kyamch is situated at a Latitude 24°19’10.9” N and Longitude 89°41’51.7” E. It is a pond near River Jamuna in Sirajganj. This pond was selected since it is a pond near the river Jamuna.

Figure 6: Name of place: Kyamch Pond Sirajganj beside river Jamuna

3.2.2. Outcome of E3: Selected Dilution concentration was 10^ -6 for this sample.

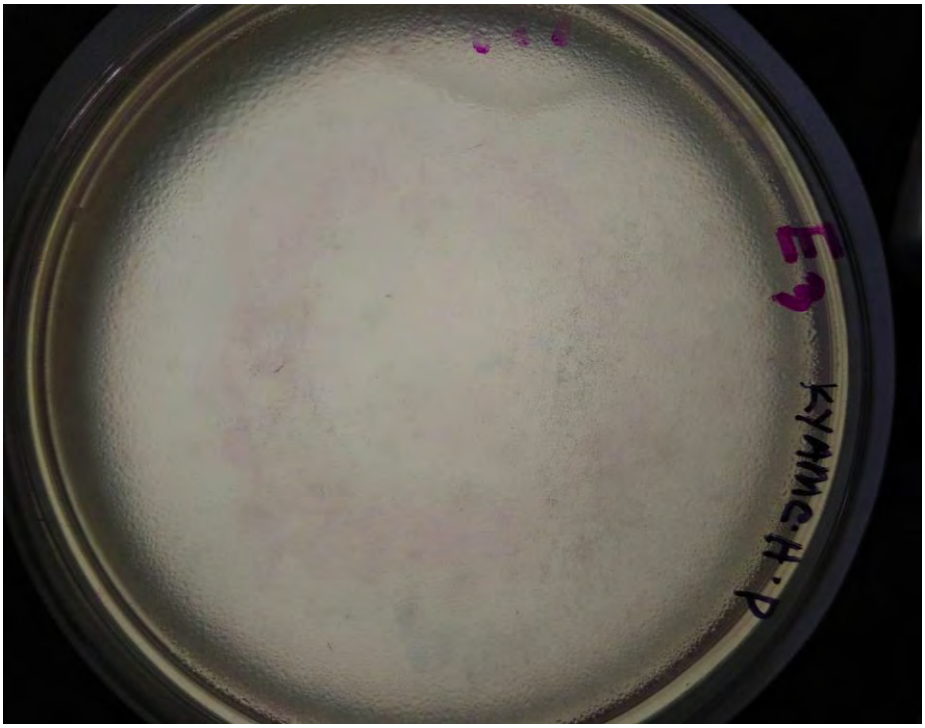

Figure 7 : Plate of E. coli ATCC from sample Kyamch pond

Results: the plate is done using E3 strain from kyamch pond. The plate is clear and no zone of inhibition is observed in the plate, which means there are no phage present that could kill the E3 strains used. Therefore, the result is negative.

Outcome of E. coli ATCC: Selected Dilution concentration was 10^ -6 for this sample.

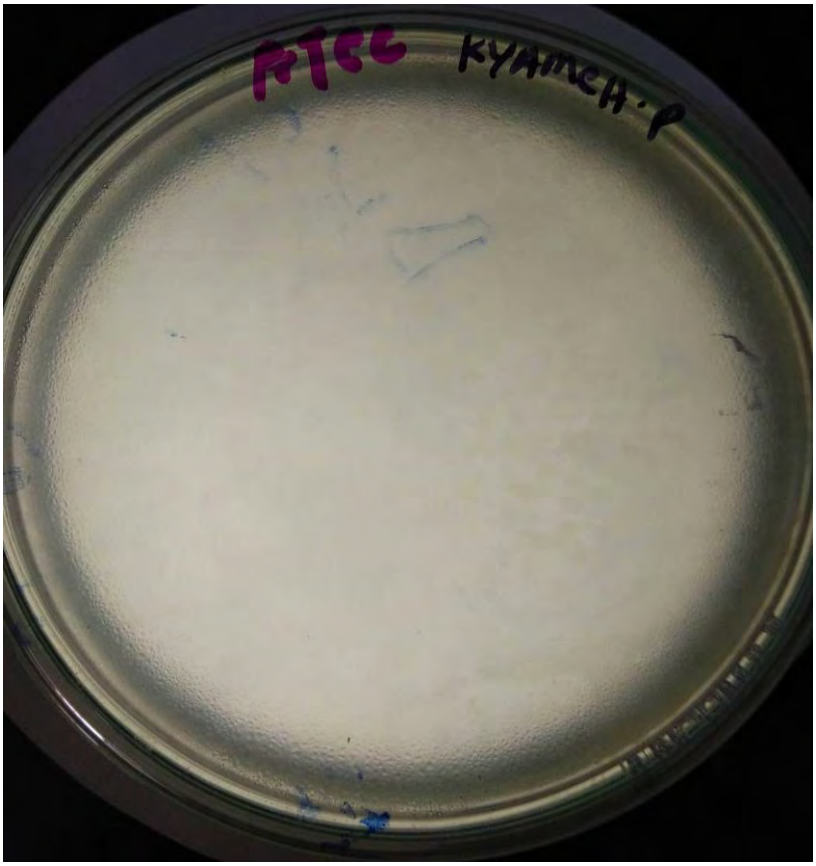

Figure 8: Plate of E. coli ATCC from sample Kyamch pond

Results: The above plate is clear; no zone of inhibition is seen in the plate which means there are no bacteriophage present in the collected sample that could kill the E. coli ATCC strain when tested once.

3.3. Sample 3:

3.3.1. Place of collection:

This pond is also situated in Sirajganj having Latitude 24°19’17.5” N and Longitude 89°41’14.1” E. This pond was selected because it is near a hospital and hospital wastes are also thrown in the pond, which makes it a good source of bacteria and phage residence.

Figure 9: Name of place: Hospital Para Pond Sirajganj

3.3.2. Outcome of E. coli ATCC: Selected Dilution concentration was 10^ -6 for this sample.

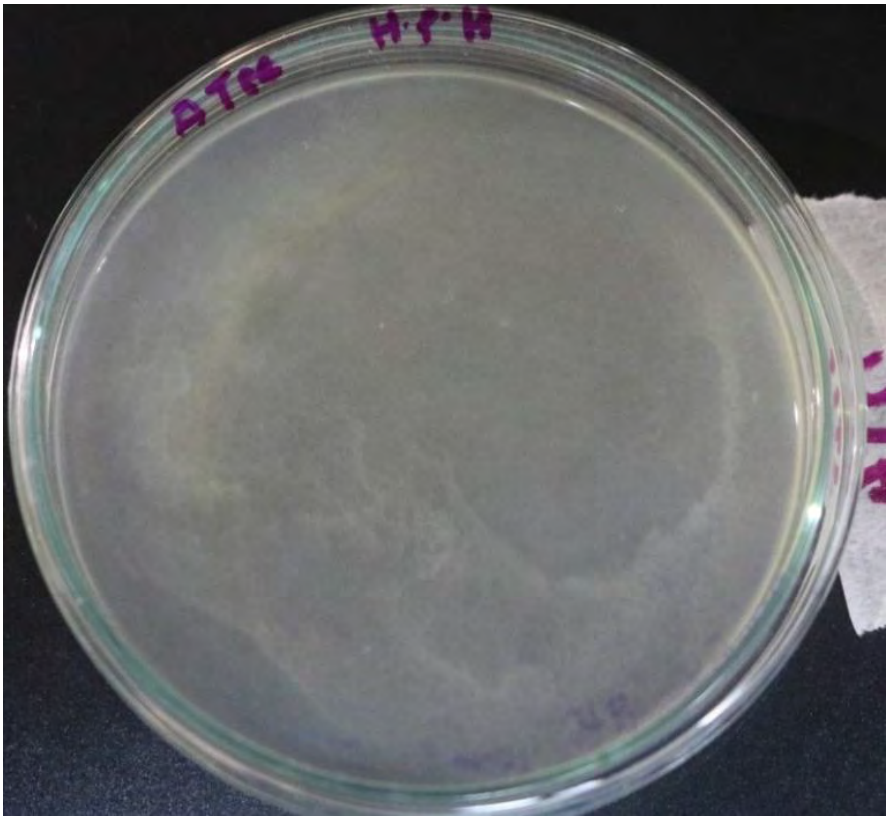

Figure 10: Plate of E. coli ATCC from sample Hospital para pond

Results: The above plate is a plate of E. coli ATCC done from hospital para pond of Sirajganj. The plate seems clear and no zone of inhibition is seen. Therefore, the result is negative and does not contain any phage that could kill the bacterial strain.

Outcome of E3: Selected Dilution concentration was 10^ -6 for this sample.

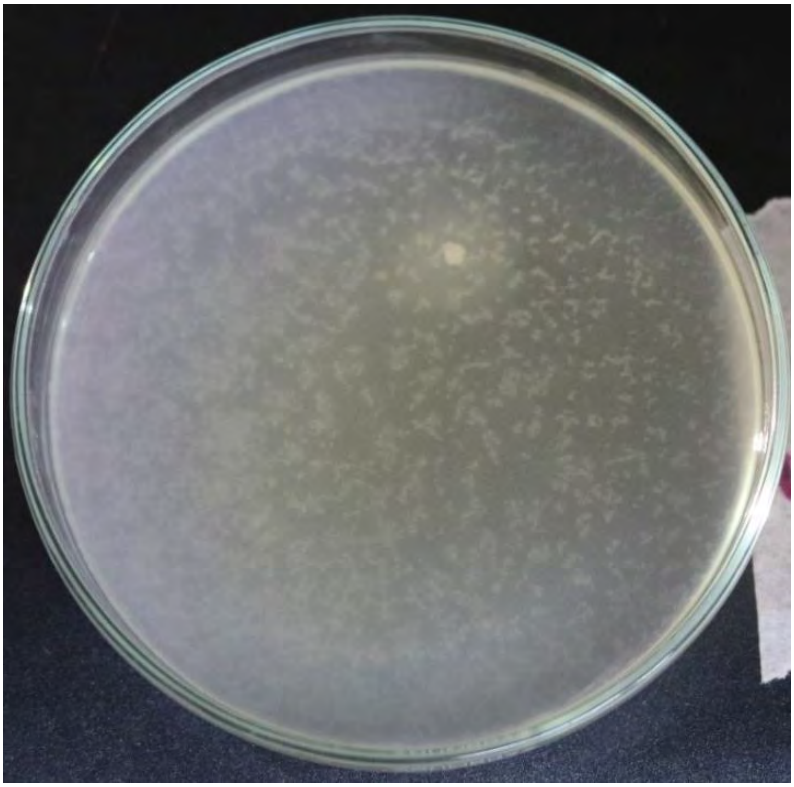

Figure 11: Plate of E3 from sample Hospital para pond

Results: In case of E3, there is a spot of contamination seen on the plate which could be during incubation from any external substance or due to moisture from the environment while experimenting. Otherwise there were no presence of phage or zone of inhibition is seen on the plates from the collected sample while tested once.

3.4. Sample 4

3.4.1. Place of collection:

This sample is collected from a lake or dighi called Nanua situated at a Latitude 23°45’91.74” N and Longitude 91°18’90.40” E situated in Comilla. This has less water stream which makes it a good source for bacteriophage residence.

Figure 12: Name of place: Nanua dighi Comilla

3.4.2. Outcome of E. coli ATCC: Selected Dilution concentration was 10^ -6 for this sample.

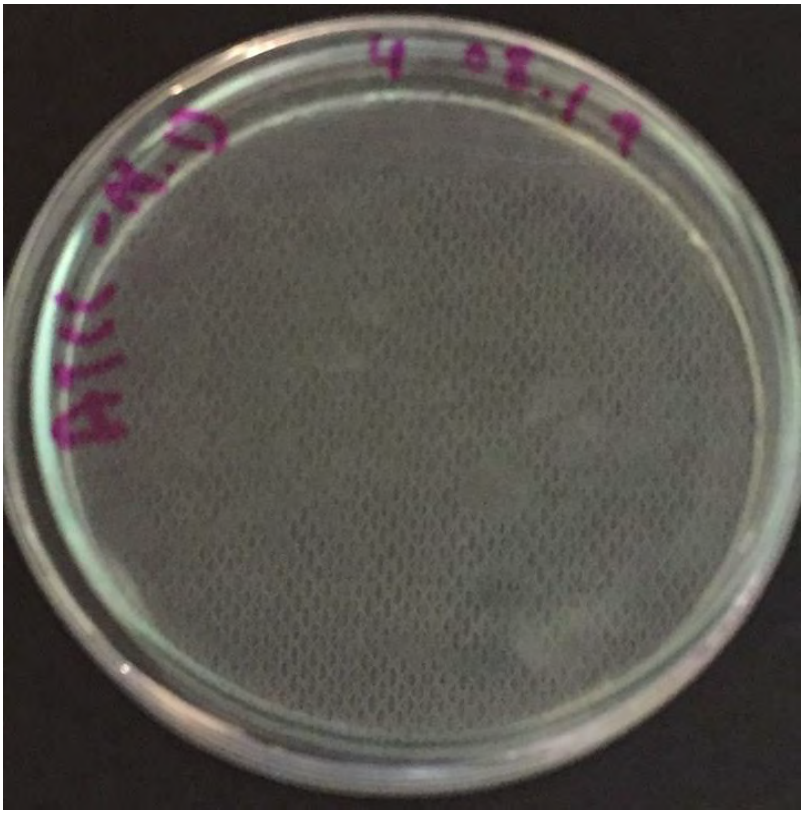

Figure 13: Plate of E. coli ATCC from sample Nanua dighi

Results: the above plate is a plate of E. coli ATCC from sample Nanua dighi. There is no zone of inhibition seen on the plate. The plate is clear and no trace of phage present. Therefore, the result is negative.

Outcome of E3: Selected Dilution concentration was 10^ -6 for this sample.

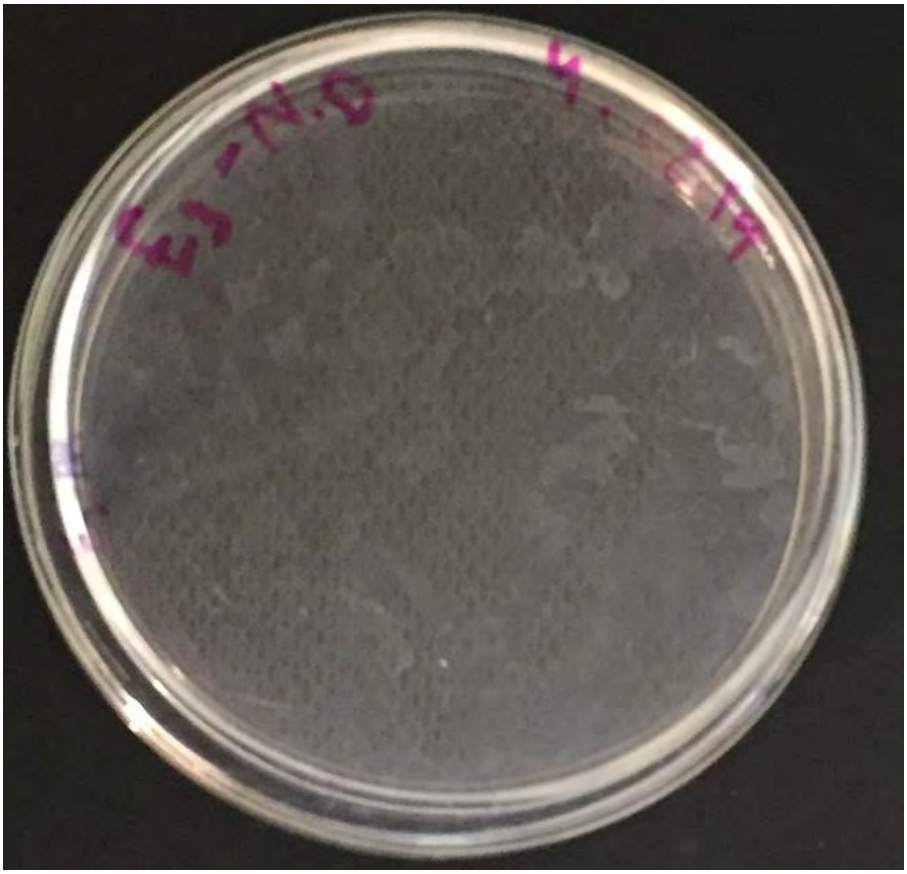

Figure 14: Plate of E3 from sample Nanua dighi

Results: there are no zone of inhibition present on the plate when E3 strain was used for sample Nanua dighi. Therefore, the result is negative.

3.5. Sample 5

4.5.1. Place of collection:

Rani dighi is situated at a Latitude 23°45’93.42” N and Longitude 91°18’30.57” E of Comilla. This lake or dighi is a small area of environmental water which contains large amount of bacteriophage.

Figure 15: Name of place: Rani dighi Comilla

3.5.2. Outcome of E. coli ATCC: Selected Dilution concentration was 10^ -8 for this sample.

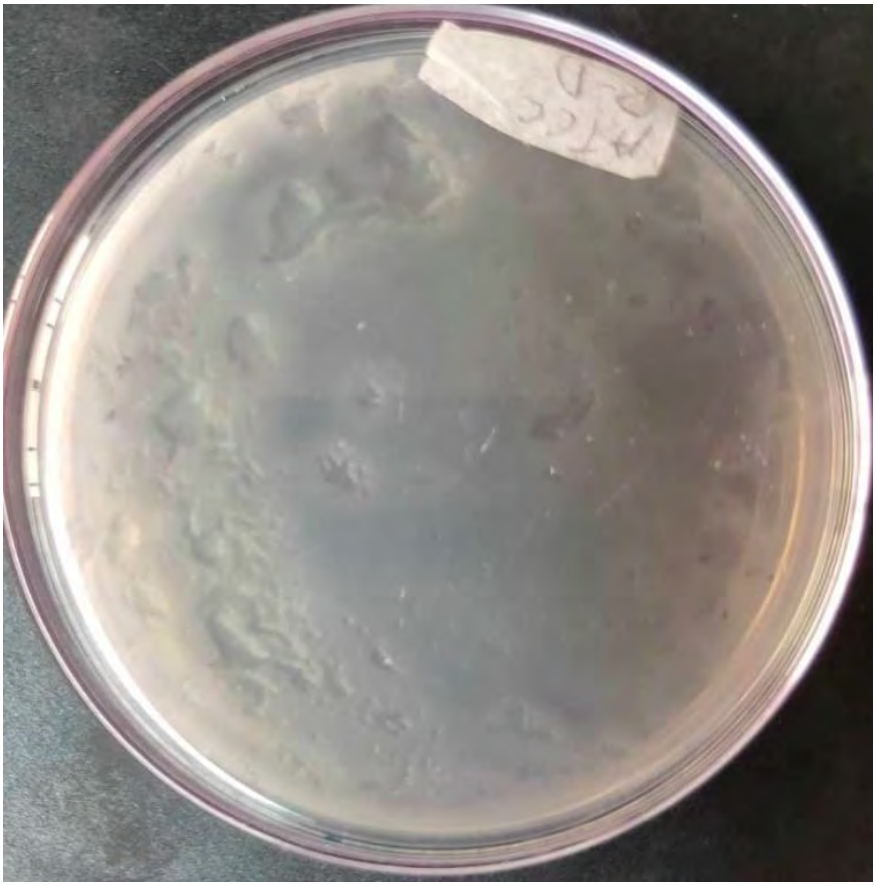

Figure 16: Plate of E. coli ATCC from sample Rani dighi

Results: the plate does not contain any plaque and no trace of zone of inhibition. Therefore, the result is negative

Outcome of E3: Selected Dilution concentration was 10^ -8 for this sample.

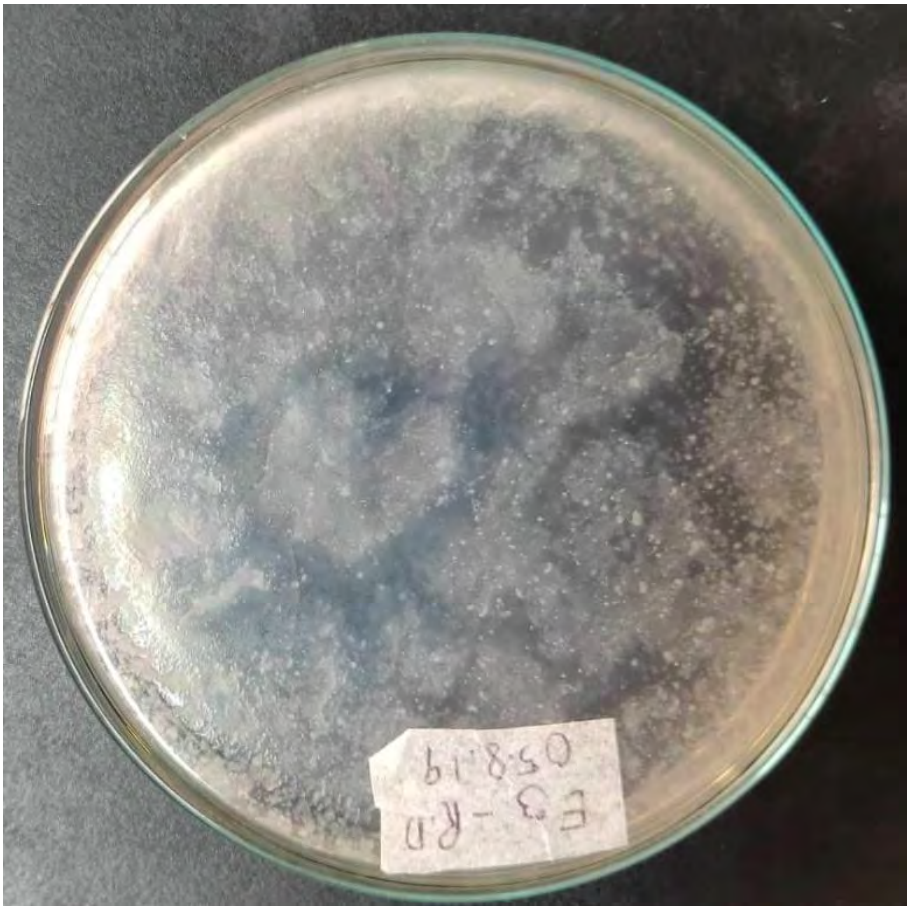

Figure 17: Plate of E3 from sample Rani dighi

Results: There is no clear zone of inhibition which means there is no plaque present which could inhibit growth of bacterial strains. The plate might contain contamination which made the plate hazy.

3.6. Sample 6:

3.6.1. Place of collection

This sample was collected from a lake called Jubilee tank situated at a Latitude 23.6023 ° N and Longitude 89.8381 °E. Since it is a small reservoir of environmental sample, therefore there could be a large scale of phage present in there.

Figure 18: Name of Place-Jubilee tank, Faridpur

3.6.2: Outcome of E3: Selected Dilution concentration was 10^ -4 for this sample.

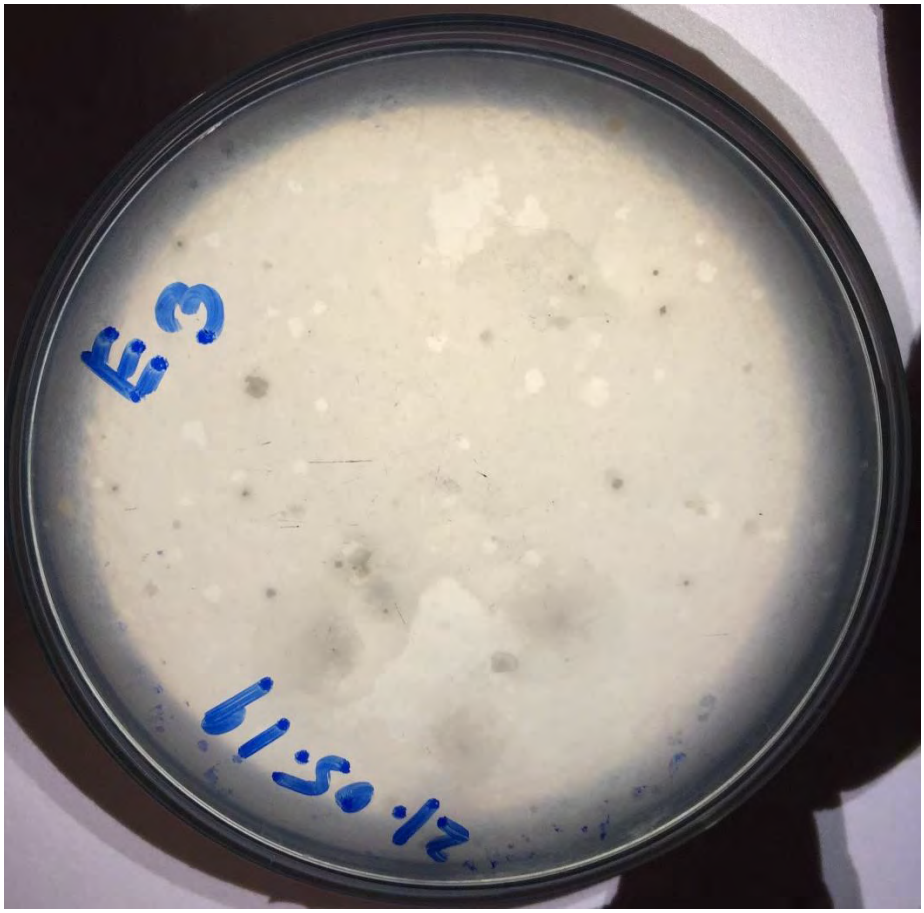

Figure 19: Plate of E3 from jubilee tank

Results: There are no zone of inhibition seen on the plate which ensures absence of bacteriophage against E3 strain.

Outcome of E. coli ATCC: Selected Dilution concentration was 10^ -4 for this sample.

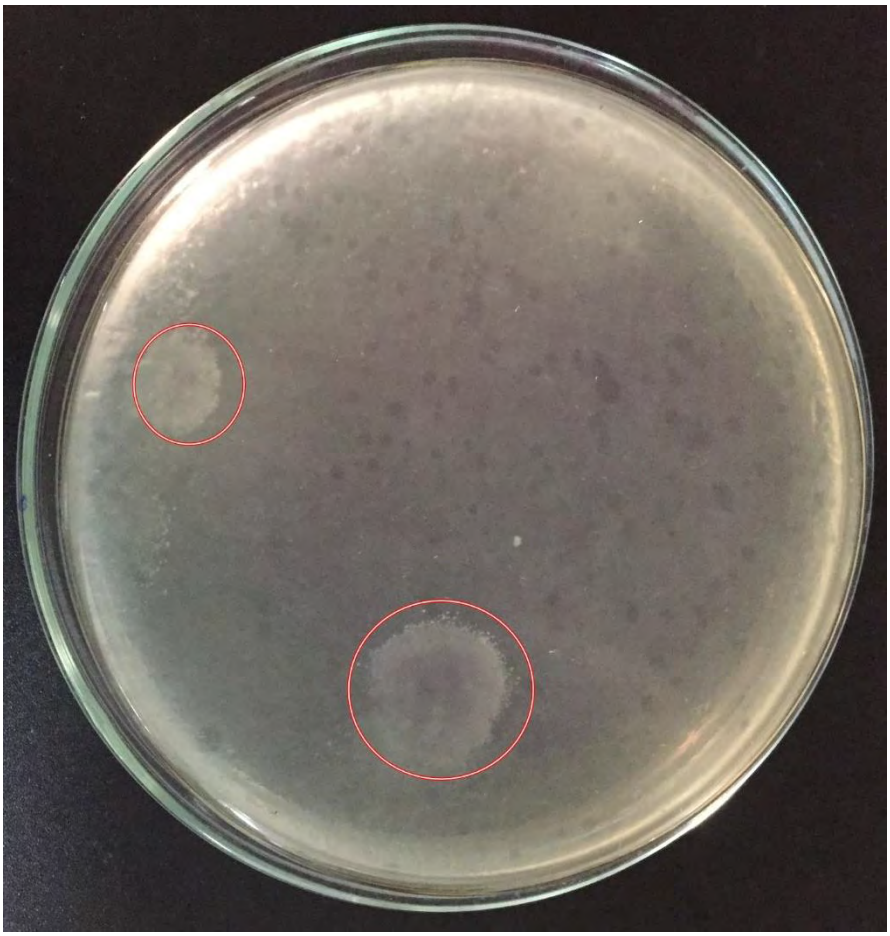

Figure 20: Plate of E. coli ATCC from sample jubilee tank

Results: In the above plate there are some very tiny clear zone of inhibition is observed and marked red. These 2 marked area is supposed to be contamination caused by water.

3.7. Samples Tested Without Dilution with Laboratory E. Coli Strain

3.7.1.1. Sample 7:

The sample is taken from another river of Bangladesh Padma situated at a Latitude 23.4662° N and Longitude 90.2897°E. The sample was collected from Paturia ghat of Padma river.

Figure 21: Name of place- River Padma (paturia ghat)

3.7.1.2: Results:

The plate is clear and there is no trace of any zone of inhibition on the plate. Therefore, there are no bacteriophage isolated from the collected amount of water from the sample.

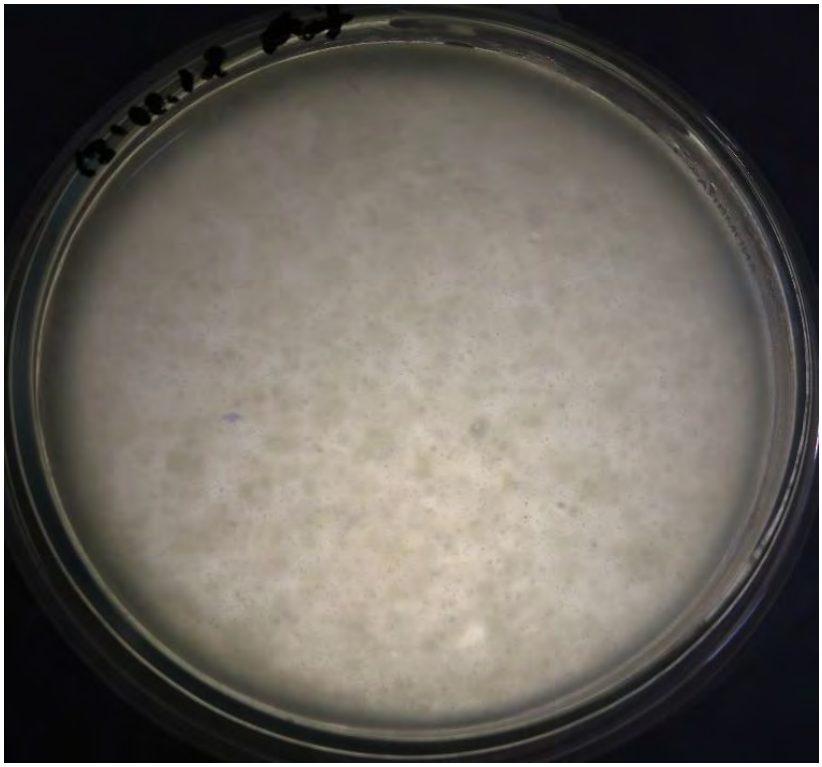

Figure 22: Plate without dilution of phage from river Padma

3.7.2.1. Sample 8: The sample was collected from IPH pond mohakhali situated at a Latitude 23.776910 ° N and Longitude 90.401712 °E. This pond is in Mohakhali area where wastes from residential area and also Icddrb hospital is thrown.

Figure 23: Name of place-IPH pond mohakhali

3.7.2.2: Results:

The plate is clear and there is no zone of inhibition seen on the plates. Therefore, there are no bacteriophage isolated from the collected amount of water from the sample.

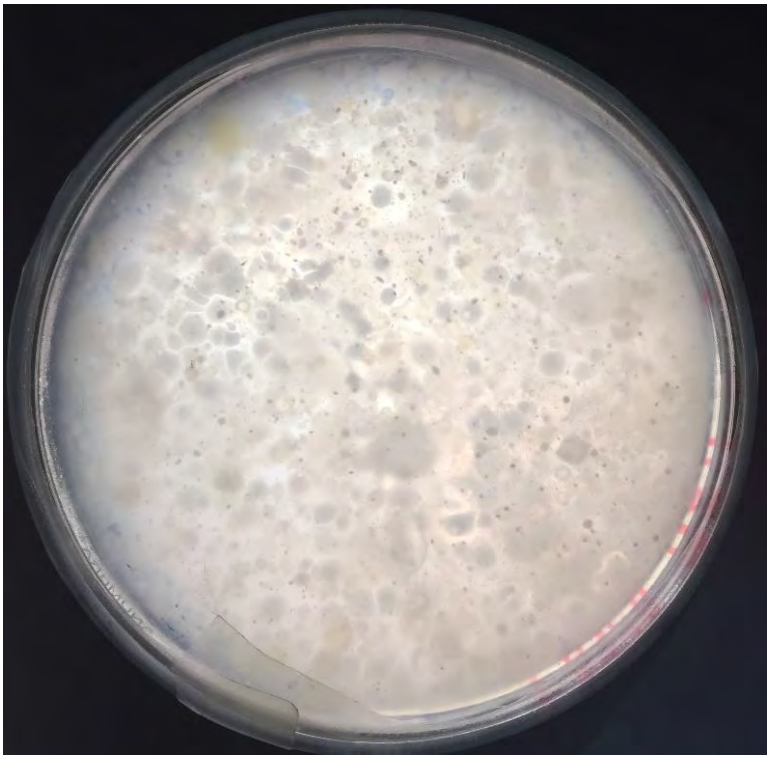

Figure 24: Plate without dilution of phage from IPH Pond

3.7.3.1. Sample 9: The sample is collected from a Latitude 23.587595 ° N and Longitude89.857616 °E. It is a lake beside Arambag Park in Faridpur.

Figure 25:Name of place-Arambag park pond, Faridpur

3.7.3.2: Results:

There is no zone of inhibition found on the plate. Therefore, there are no bacteriophage isolated from the collected amount of water from the sample.

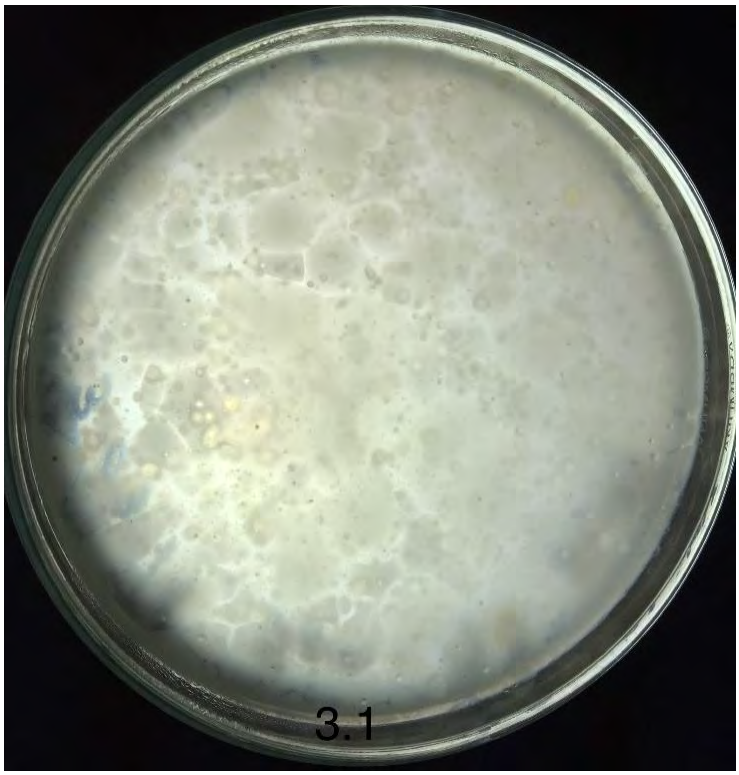

Figure 26: Plate without dilution of phage from Arambag Park Pond, Faridpur

3.7.4.1. Sample 10:

The sample is collected from a place having Latitude 23.776049°N and Longitude 90.397193 °E in Narshindi. Since a pond is a good source for phage residence therefore this pond was chosen for isolation.

Figure 27: Name of place-Narshindi

3.7.4.2: Results:

There is no zone of inhibition found on the plate. Therefore, there are no bacteriophage isolated from the collected amount of water from the sample.

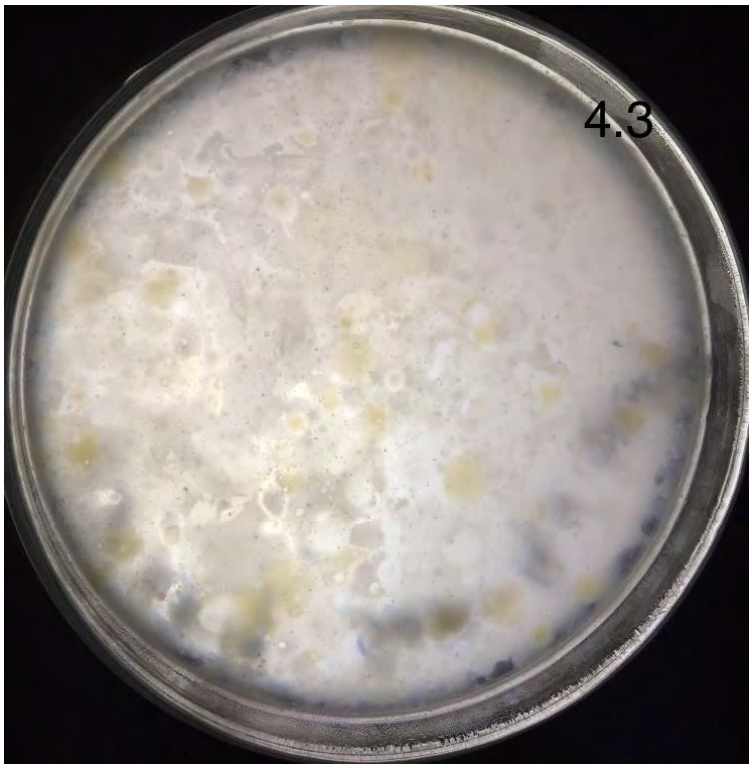

Figure 28:Plate without dilution of phage from Narshindi

Table 2. 3.8. Result Overview

Place of collection	latitude	longitude	Date of collection	Bacterial strain	dilution	Results
Buriganga	23°37’59.99” N	90°25’59.99” E	25.06.19	E3, ATCC	10^-6	Negative
Kyamch	24°19’10.9” N	89°41’51.7” E	13.7.19	E3, ATCC	10^-6	Negative
Hospital para	24°19’17.5” N	89°41’14.1” E	19.7.19	E3, ATCC	10^-6	Negative
Nanua dighi	23°45’91.74” N	91°18’90.40” E	6.07.19	E3, ATCC	10^-6	Negative
Rani dighi	23°45’93.42” N	91°18’30.57” E	6.07.19	E3, ATCC	10^-8	Negative
Jubilee tank	23.6023 ° N	89.8381 °E	15.05.19	E3, ATCC	10^-4	Positive
IPH pond	23.776910 ° N	90.401712 °E	10.03.19	E. coli	Not done	Negative
Arambag	23.587595 ° N	89.857616 °E	22.02.19	E. coli	Not done	Negative
Narshindi	23.776049° N	90.397193 °E	7.03.19	E. coli	Not done	Negative
Padma	23.4662° N	90.2897°E	10.02.19	E. coli	Not done	Negative

Figure: Overview of total 10 samples

It is to be noted that all the samples were collected once at a particular amount and tested once.

Chapter:4

Discussions

Out of 10 collected samples only 1 sample could show some traces of bacteriophage at concentration 10^-4. However, the traces of phage were guessed by clear plaque formations on the plate and those were decided to be considered for further testing for confirmation and then isolation.

The other samples did not show such occurrence of plaques. There was no clear zone on the plates that would confirm presence of phage. But for confirmation of plaque absence, the sample should be tested 2 other times, in total of 3 times for each sample.

There could be many reasons of the absence of plaque, such as it is necessary to collect a very large quantity of sample from environmental sources at various seasons and should be tested individually for 3 times to confirm it (Jo & Zaczek, 2016). But due to laboratory unavailability of schedule and sometimes due to unavailability of getting schedule of machineries and constrain of time, sample could only be tested once. Also large amount of sample was not collected. Only a full of Duran bottle which has a capacity of 250ml was collected, that is a very small amount as compared to the source. However, many of the samples were collected during rainy reason, therefore there is a chance of the phages to get diluted by rain water. The presence of phage may differ due to seasonal variation.

In laboratory, the problem may also be in nutrient agar and nutrient broth that are available in the lab, since it is used by many and contamination might have occurred.

Therefore, if the chance to reexamine the samples is possible, it is highly hoped to get positive results in future.

Chapter :5

Conclusion:

The main aim of the experiment was to detect presence of phage and the isolate them for further characterization, since bacteriophage is a recent scope of therapeutic progress in a developing country like Bangladesh. If the isolation is near future, antibiotic resistance among the people of the country could be minimized through using phage therapy. However, few samples showed criteria of plaque which needs further confirmation through using large volume of environmental samples. Therefore, proper isolation criteria, time and facilities should be provided to do so.

Chapter:6

References

A, v. K. (1999). Beijerinck's contribution to the virus concept--an introduction. *NCBI*.

agata Jurczak-Kurek, T. G.-F.-D. (04 October 2016). Biodiversity of bacteriophages: morphological and biological properties of a large group of phages isolated from urban sewage. *Nature*.

Bacteriophages. (n.d.). *khan academy*.

Bacteriophages: Structure and Reproduction (Replication Cycle). (n.d.). Retrieved from Biology discussion : http://www.biologydiscussion.com/viruses/bacteriophages-structure-and-reproduction-replication-cycle/5690

Bhandari, I., Colet, E., Parker, J., Pines, Z., Pratap, R., & Ramanujam, K. (1997, March). Advanced Scout: Data Mining and Knowledge Discovery in NBA Data. *Data Mining and Knowledge Discovery*, 121-125.

Brian, S. (2012, January 25). The Problem of Shot Selection in Basketball. *PLoS One*. Retrieved from https://doi.org/10.1371/journal.pone.0030776

Britannica, T. E. (2019 updated, june 16). *Dmitry Ivanovsky*. Retrieved from Encyclopædia Britannica: https://www.britannica.com/biography/Dmitry-Ivanovsky

Choudhury, R. D., & Bhargava, P. (2007). Use of Artificial Neural Networks for Predicting the Outcome. *International Journal of Sports Science and Engineering, 1*(2), pp. 87-96.

Classification of Bacteriophage | Bacterial Virus. (n.d.). Retrieved from Biology discussion: http://www.biologydiscussion.com/viruses/classification-of-bacteriophage-bacterial-virus/49987

Duckworth, F., & Lewis, T. (1999). *Your Comprehensive Guide to the Duckworth/Lewis Method for Resetting Targets in One-day Cricket.* University of the West of England.

Garcia, H. (2018). Louis Pasteur And The Germ Theory Of Disease. *StMU History Media*.

Kasman, L. M., & Porter., L. D. (january 2019). Bacteriophage. *NCBI*.

Kenneth Todar, P. (2008-2012). Bacteriophage. *Text book of Bacteriology*, 2.

kenneth Todar, P. (n.d.). Bacteriophage. *Online Textbook Of Bacteriology*, 1.

Mohamed Elbreki, R. P. (26 March 2014). Bacteriophages and Their Derivatives as Biotherapeutic Agents in Disease Prevention and Treatment. *Journal of Viruses*.

N., S. (n.d.). *Bacteriophage.* Retrieved from Biology reader: https://biologyreader.com/bacteriophage.html#Structure

Sankaranarayanan, V. V., Sattar, J., & Lakshmanan, L. S. (2014). Auto-play: A data mining approach to ODI cricket simulation and prediction. *SIAM International Conference on Data Mining*, (p. 1064).

Shah M. Faruque, I. B. (2005). Seasonal epidemics of cholera inversely correlate with the prevalence of environmental cholera phages. *PNAS*.

Shah M. Faruque, N. C. (2003). Shigella dysenteriae Type 1-Specific Bacteriophage from Environmental Waters in Bangladesh. *American Society For Microbiology*.

The Discovery of Viruses. (n.d.). *Encyclopedia.com-Encyclopedias almanacs transcripts and map*.

Tulabandhula, T., & Rudin, C. (2014). Tire Changes, Fresh Air, and Yellow Flags: Challenges in Predictive Analytics for Professional Racing. *Big data*. Retrieved from http://doi.org/10.1089/big.2014.

Chibani-chennoufi, S., Bruttin, A., Brüssow, H., Dillmann, M., & Bru, H. (2004). Phage-Host Interaction : an Ecological Perspective MINIREVIEW. *J. Bacteriol.*, *186*(12), 3677–3686. https://doi.org/10.1128/JB.186.12.3677

Criscuolo, E., Spadini, S., Lamanna, J., Ferro, M., & Burioni, R. (2017). Bacteriophages and Their Immunological Applications against Infectious Threats. *Journal of Immunology Research*, *2017*. https://doi.org/10.1155/2017/3780697

Haq, I. U., Chaudhry, W. N., Akhtar, M. N., Andleeb, S., & Qadri, I. (2012). □□□□□□□ *«2»*. 1–23.

Huang, C., Virk, S. M., Shi, J., Zhou, Y., Willias, S. P., Morsy, M. K., … Li, J. (2018). Isolation, characterization, and application of Bacteriophage LPSE1 against Salmonella enterica in Ready to Eat (RTE) Foods. *Frontiers in Microbiology*, *9*(MAY), 1–11. https://doi.org/10.3389/fmicb.2018.01046

HUDSON, J. A., BILLINGTON, C., CAREY-SMITH, G., & GREENING, G. (2016). Bacteriophages as Biocontrol Agents in Food. *Journal of Food Protection*, *68*(2), 426–437. https://doi.org/10.4315/0362-028x-68.2.426

Jo, E., & Zaczek, M. (2016). *Bacteriophage Procurement for Therapeutic Purposes*. *7*(August), 1–14. https://doi.org/10.3389/fmicb.2016.01177

Keen, E. C. (2015). A century of phage research: Bacteriophages and the shaping of modern biology. *BioEssays*, *37*(1), 6–9. https://doi.org/10.1002/bies.201400152

Moelling, K., Broecker, F., & Willy, C. (2018). A wake-up call: We need phage therapy now. *Viruses*, *10*(12), 1–14. https://doi.org/10.3390/v10120688

Naser, I. Bin, Hoque, M. M., Abdullah, A., Bari, S. M. N., Ghosh, A. N., & Faruque, S. M. (2017). Environmental bacteriophages active on biofilms and planktonic forms of toxigenic Vibrio cholerae: Potential relevance in cholera epidemiology. *PLoS ONE*, *12*(7), 1–15. https://doi.org/10.1371/journal.pone.0180838

Pelzek, A. J., Schuch, R., Schmitz, J. E., & Fischetti, V. A. (2013). Isolation, Culture, and Characterization of Bacteriophages. In *Current Protocols in Essential Laboratory Techniques* (Vol. 2013). https://doi.org/10.1002/9780470089941.et0404s07

Plotkin, S. (2014). *History of vaccination*. *111*(34), 12283–12287. https://doi.org/10.1073/pnas.1400472111

Terms, F. (2016). *a n d e s i o s c i e n c e o n o t d i s t r i b u t e*. *7081*(March). https://doi.org/10.4161/bact.1.3.16736

Appendix A.

INGREDIENTS AND DISHES:

Bacterial host strains

Bacterial growth medium: 1. Nutrient agar broth (liquid) 2. Nutrient agar (solid)

Bacteriophage liquid stock (used after filtration and centrifugation)

Phosphate buffer saline: PBS

Sterile Duran bottles: for collection of water samples

Filter paper: for sample filtration

Micro filters or syringe filters: 0.22 μm size

Beakers (autoclaved)

Vials: 5ml size (autoclaved)

Sterile cotton

70% ethanol solution

90mm petri dishes (autoclaved)

Test tubes (autoclaved)

Conical flasks (autoclaved)

Aluminum foil

INSTRUMENTS:

Incubator (used at 37 degrees Celsius for bacterial growth)

Refrigerator: for storage

Laminar flow: to ensure sterile environment while working

Shaking incubator

Centrifuge

Autoclave

Water bath (capable of heating 30-70 degrees Celsius)