Correlation between the prevalence of plasmids and AMR genes in the environmental isolates of different bacterial species

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A Dissertation Submitted to BRAC University in Partial Fulfillment of the Requirements for The Degree of Master of Science in Biotechnology

Department of Mathematics and Natural Sciences

BRAC University

July 2023

Declaration

It is hereby declared that

1. The thesis submitted is my 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 that has been accepted, or submitted, for any other degree or diploma at a university or other institution.

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Acknowledgement

First and foremost, I would like to express my thanks to almighty Allah because He has given me the opportunity, and strength to finish this research. I am also thankful for His blessings to my daily life, good health and healthy mind.

I acknowledge my esteem to Professor **A F M Yusuf Haider**, Chairperson of MNS Department, and Associate Professor **Dr. Munima Haque**, Coordinator of the Biotechnology Program of MNS Department of BRAC University for allowing me and encouraging me to complete my undergraduate thesis.

My regards, gratitude, indebtedness, and appreciation go to my respected supervisor **Dr. Iftekhar Bin Naser***,* Assistant Professor, Department of Mathematics and Natural Sciences, BRAC University for his constant supervision, constructive criticism, expert guidance, enthusiastic encouragement to pursue new ideas and never ending inspiration throughout the entire period of my research work. I would like to thank and express my deepest gratitude for guiding me in my report writing and providing time to time suggestions regarding the setting of my experimental designs, interpretation of results, and subsequence directions for the whole work without being a bit of impatient. It would have been impossible to submit my report without his cordial help.

I would like to extend my appreciation to the respective Lab officers Asma Binte Afzal, Mahmudul Hasan, Research Assistant Rockybul Hasan, for their suggestions and moral support during my work.

Finally, I would like to extend my gratitude to the members of my family and friends for their prayerful concerns and supports.

Wasifa Ar Rahman

July 2023

Abstract

Bacterial gene transfer is responsible for spread of antibiotic resistance in bacteria and evolution of bacteria which can be a threat of virulence as well play role in maintenance. Horizontal gene transfer (HGT) is the key process for transferring gene. It involves three mechanisms- conjugation, transformation and transduction. To distantly related species of bacteria, via HGT, exogenous DNA can be transferred from one bacterium to another. Apart from HGT, prophage induction and infection can be a way of transferring gene. For this, the prophage is induced by UV radiation and infects with bacterial competent cell to make the new viral particles. On the infection with other bacteria, the prophage can carry the gene through plasmid packaging or miss packaging and may also be integrated to the chromosomal DNA of the newly formed particle. Following that, in preliminary selection it showed growth of resistant colonies of its bacteria insuring of gene transfer. Further for genetic validation, plasmid isolation assures of plasmid packaging, here the result was negative for plasmid packaging thus it indicates of gene transferring and integrating to the chromosomal DNA. To validate the result, more advanced study and farther genetic validation is required like polymerase chain reaction to assure the presence of the antibiotic resistant gene in the infected particles.

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CHAPTER 1
INTRODUCTION

1.1 Bacterial Extra Chromosomal DNA- "Plasmid"

Bacterial plasmid is a form of small circular DNA molecule which are physically separate from chromosomal DNA and replicate independently. They normally contain only a few genes, including some linked to antibiotic resistance, and they can pass from one cell to another (NIH 2023).

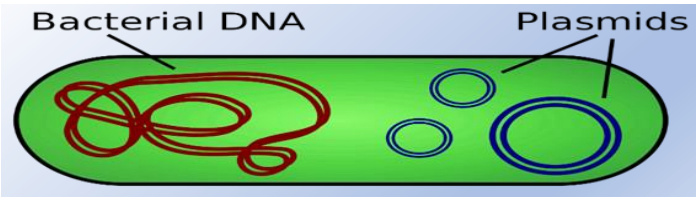

Figure 1.1: Bacteria containing chromosomal DNA and plasmid ([1]Adapted From- Wikipedia- Plasmid n.d.)

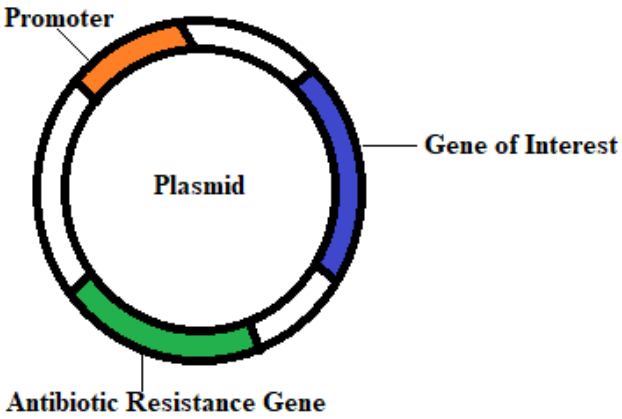

Figure 1.2: Elements of bacterial plasmid ([2]Adapted From- Khan Academy- Bacterial transformation & selection n.d.)

1.2 Bacterial Plasmid Isolation from River and Lake Water

By using alkaline or enzyme lysis and agarose gel techniques, bacteria isolated from groundwater aquifer core materials of pristine aquifers at Lula and Pickett, OK, and from a site with a history of aromatic hydrocarbon contamination and natural renovation located at Conroe, TX, were screened for the presence of plasmid DNA.[3] In this research, bacteria isolated from lake water were screened for the presence of plasmid DNA by alkaline lysis technique and later assured by gel electrophoresis. Lake waters are polluted water which had a significant number of plasmids containing bacteria. In accordance [4]from the silt of unpolluted and polluted sites in a swiftly flowing south Wales River, they isolated 400 aerobic heterotrophic bacteria. Taxonomic assays were performed on the isolates, and plasmid DNA was checked for using agarose gel and alkaline

lysis methods. Both the overall percentage of isolates with plasmids (unpolluted site, 9.4%; polluted site, 15%) and the percentage of non-Pseudomonas-like isolates with plasmids (unpolluted site, 15%; polluted site, 10%) did not differ significantly between the sites. In this research, from the lake water bacteria plasmids were significantly identified for *Escherichia coli* (E. coli) bacteria, *Shigella* and *Vibrio cholera.* The plasmid number varied for the respective bacteria.

1.3 Bacterial Gene Transfer

Since the controversy over the possible threats posed to the environment by the accidental or deliberate release of genetically engineered organisms, the horizontal transmission of genetic material across bacteria in microbial ecosystems has drawn considerable attention.[5] In accordance of [6] Levy and Miller-1989 prokaryotic gene transfer is a system that is relatively open: In close contact between cells (conjugation), DNA can be transferred via phages (transduction) or released naturally by live cells for future cellular uptake. Specific tools, called conjugative pili and phage virions, are used during transduction and conjugation to transfer DNA from donor to recipient cells.[7]

1.4 Horizontal Gene Transfer

Long-term bacterial evolution and environmental adaptation are both facilitated through horizontal gene transfer (HGT) across bacterial cells.[8] However, it results in the undesirable spread of pathogenicity, antibiotic resistance, or genetically modified organisms in human environment.[9]

One method of horizontal gene transfer (HGT) in bacteria is transformation, when cells with genetic competence take up extracellular naked DNA. The ability of transformation to be distinguished from DNase-resistant HGT processes depends on their susceptibility to DNase, which breaks down bare DNA. In general, transformation is primarily a function of recipient cells that express competence to take up extracellular naked DNA.[10]

Transformation competence can be actually or artificially induced, but not all bacterial species develop natural competence.[11] In normally transformable microbes, competence is more often than not temporal and initiated by modifications within the development state of living being.[12] A gather of “competence genes” has been recognized, and common mechanistic models have been proposed,[13] in spite of the fact that exact components for person bacterial species have not been

adequately illustrated.[14] Since transformation requires extracellular naked DNA as the substrate, sensitivity to DNase, which degrades naked DNA, is key in recognizing change from other DNase-resistant HGT mechanism.[15]

The horizontal transfer of an altered PBP2B gene from penicillin-resistant *S. pneumoniae* into *S. sanguis* is thought to be how penicillin resistance first emerged. *Streptococcus pneumoniae* strains with lower penicillin affinities have changed forms of the penicillin-binding proteins (PBPs).[16] The development of penicillin resistance in pneumococci shows up to have included the replacement, on at least two events, apparently by change, of parts of the initial pneumococcal PBP2B gene with the homologous regions from closely related species.[17]

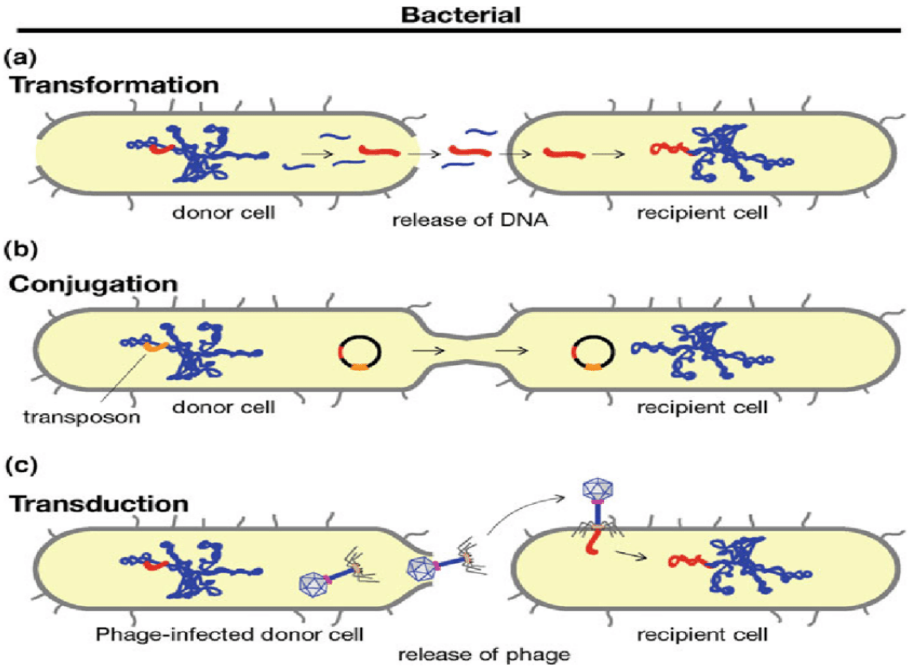

Figure 1.3: The methods of horizontal gene transfer in bacteria (Adapted with permission from-[18]Vernikos and Medini 2014; copyright 2014 Springer Nature.)

1.5 Prophage Induction & UV Radiation

Physical and chemical factors that change DNA replication or structure can cause prophages. If the lysogenic bacterium's DNA is transformed, the prophage is directly induced; alternatively, the prophage may be indirectly induced if altered DNA is transferred into the lysogen through conjugation, transfection, or transformation [19]or by conjugation or transfection. Prophages are induced in UV-sensitive bacteria that lack excision-repair by relatively modest UV radiation

exposures. The conclusion that the bacterial excision-repair mechanism operates to reduce the induction response has been reached as a result of the discovery that uvr+ lysogens require greater doses of UV radiation for induction.

In this study, the prophage of the bacteria is induced by UV radiation and the induced prophage are infected to produce the infected particles which are the newly formed viral particles containing the gene of its bacteria. The infection allows the transfer of gene to competent cell in order to make the new viral particles. The gene is carried by plasmid packaging to the newly formed particles or by integrating to the chromosomal DNA of the newly formed viral particles.

CHAPTER 2

MATERIALS & METHODS

2.1 Sample Collection, Bacteria Isolation, Stocking of Isolated Bacteria

Samples were collected from lake water at Dhanmondi area located at Dhaka. The lake water samples were then filtered and grown over culture for 4 hours in the composition of 6 ml Luria Broth (LB) media and 4 ml sample water. Serial dilution was done in the cultured sample then plated by spread plate method in three selective medias. The selective media used are MacConkey agar, Salmonella Shigella agar and Thiosulfate–citrate–bile salts–sucrose agar (TCBS) AGAR which promote the growth of *Escherichia coli* (E. coli) bacteria, *Shigella* and *Vibrio cholerae* respectively. After that, the single colonies grown on the selective agar media plates for the respective bacteria were stocked in 2 ml Eppendorf consisting of 1 ml Luria Agar (LA) media.

2.2 Pure Culture Preparation, Plasmid Extraction and Gel Electrophoresis

In order to get pure culture of the three isolated bacteria, from the stocks of bacteria they were grown in Luria agar plate in streak plating technique to get single colonies from 24-hour incubation (37ºC). After that, single colony was inoculated in 9 ml Luria broth and cultured for overnight (37ºC) to get the pure culture. The pure culture of bacteria cells harboring desired plasmid then used for plasmid extraction. 1.0 ml cells were transferred to harvest by centrifugation at 12000Xg for 5 minutes. Supernatant was removed, the pellet then resuspended in solution I (200 µl) and to this freshly prepared solution II (400 µl) was added and mixed by gentle inversion. The cells were incubated at room temperature for 5 minutes for cell lysis. To this, ice cold solution III (300 µl) was added, mixed by inversion and incubated on ice for 10 minutes. Then, the mixture was centrifuged at 12000Xg for 15 minutes. The supernatant was collected in a fresh tube. To this, equal volume of phenol: chloroform: IAA (25:24:1) was added, mixed by vortexing and centrifuged at 12000Xg for 2 minutes. Supernatant was collected carefully and equal volume of chloroform: IAA (24:1) was added, mixed by vortexing and centrifuged at 12000Xg for minutes. Supernatant was collected and then equal volume of isopropanol was added to this, mixed by inversion and centrifugation at 12000Xg for 15 minutes. The DNA pellet was washed in 70% ethanol and resuspended in 20 µl of Tris-EDTA buffer solution- TE buffer (10 mM Tris-HCl, 1 mM disodium EDTA, pH 8.0). Then the extracted plasmid was stored at -20ºC. After this, the extracted plasmid products were resolved in a 1.0% agarose gel ran in 1X TAE buffer (Tris-acetate-EDTA) (40mM Tris, 20mM Acetate and 1mM EDTA) for 2 hours at 90 voltages.

2.3 Prophage Induction, Competent Cell Preparation, Infection

From the stock of bacteria (*E. coli*, *Shigella* and *V. cholerae)* subculture was done. From the sub culture, single colony was taken for liquid culture preparation in 2 ml vial mixing with Luria broth. The liquid culture run overnight at 37 ºC. After then, 1 ml of the liquid culture transferred in Eppendorf and induced in UV (ultra-violet transilluminator) for 20 seconds. Then, 100 µl of UV induced culture and 2 ml of fresh Luria broth was mixed, further cultured for (5-6) hours. After the culture, the mixture was centrifuged at 12000Xg for 5 minutes. Then the supernatant was collected and filtered with 0.22 µm filter. The supernatant was stored at -20 ºC for later use. Here, the supernatant may contain some virus particle which remain in bacterial cell as prophage or as an extrachromosomal plasmid. So, by UV induction they will get out from the bacterial cell as virus containing the plasmid or the prophage as their genetic element.

Competent cell was prepared by DH5α Cells and Vibrio 404 cells. DH5α and Vibrio 404 cells were grown in overnight culture of 10 ml. After that, they were centrifuged at 5000 rpm for 5 minutes. Then, the supernatant was discarded and 1 ml of 10% glycerol was added and resuspended the pellet with it. Another centrifuge was run at 5000 rpm for 5 minutes. This step was repeated two times. Finally, the remaining pellet was dissolved in 0.5 ml of 10% glycerol.

For the infection of the collected prophage from the prepared supernatant with the prepared competent cell of DH5α and Vibrio 404, 2 Eppendorf tubes were marked with Ampicillin (AMP) and 100 µl of prepared competent DH5α cells was taken in one Eppendorf tube and other 100 µl of prepared competent Vibrio 404 cells was taken in another Eppendorf tube. Another set of 2 Eppendorf tubes were taken which contained 30 µl of collected supernatant from 10 strains of the bacteria (*E. coli*, *Shigella* and *V. cholerae).* So, the total was 300 µl for each tube. After that, the supernatant (300 µl) was added to 100 µl of DH5α and Vibrio 404 cells. Then it left for infection at 30 ºC for overnight, 2 hours and 6 hours. After the infection at the mentioned time periods, the infected samples were plated (Spread plate technique) in Ampicillin plates as raw and with a 10-1 dilution. Here, in case of plating in AMP plates the resistant phages will grow which might have carried the plasmid or the prophage as their genetic material.

CHAPTER 3

RESULTS

3.1 Sampling:

The sampling was done in over 3 months (April 2022- June 2022). In this duration, sampling was done for 10 times. From the sampling, isolated three bacteria *Escherichia coli* (E. coli), *Shigella* and *Vibrio cholera.* 75 of the stocking was done for these isolates.

3.2 Antibiogram of The Isolates:

From the stock antibiogram was done for the bacterial isolates. Three antibiotic discs (Ampicillin- AMP 30 µg, Tetracycline- TE 30 µg, Kanamycin- K 30 µg) were used and measured with CLSI published antibiotic sensitivity standard chart.

The antibiogram result is shown in a graph given below:

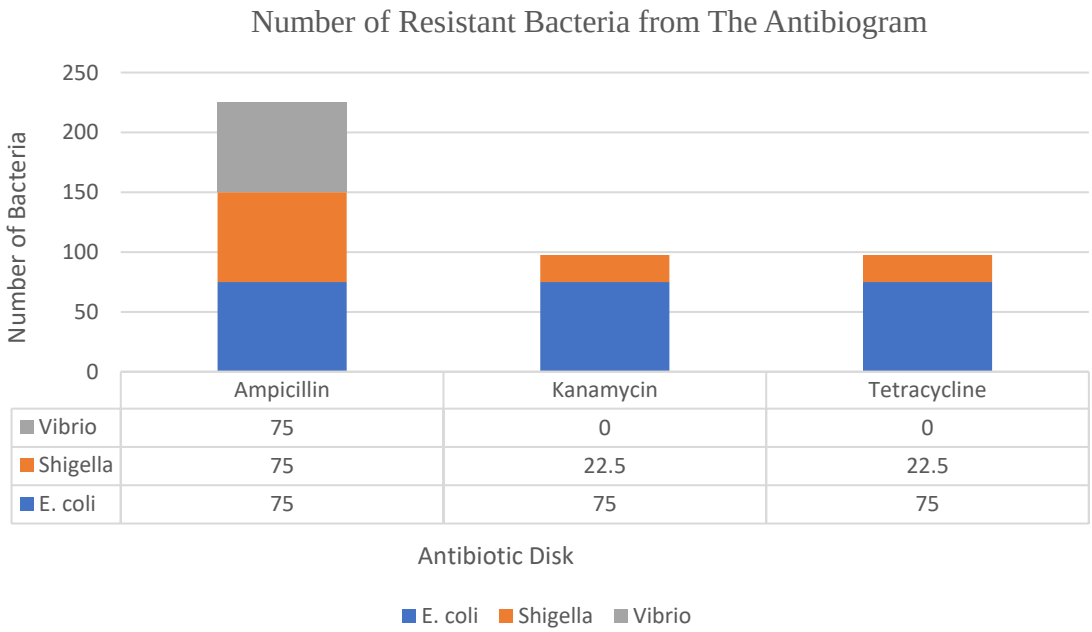

Figure 3.1: Number of resistant bacteria from antibiogram result

As a result, I have got many of the bacteria are resistant for almost all of the antibiotics. So, it indicates that as the Dhanmondi, Dhaka area have a huge number of hospitals, therefore, the bacteria from the lake water of this area hold a great number of antimicrobial resistant (AMR) genes.

3.3 Plasmid Profiling:

From the 75 isolates of stock, 60 isolates were taken for plasmid profiling. Among this, 45 isolates showed greater resistance (resistant in almost all of the antibiotics) in antibiogram and 15 isolates showed less resistance (sensitive in most antibiotics) in antibiogram.

The result of the plasmid profiling is given below:

Table 1: Plasmid profiling of 45 isolates showing greater resistance to antibiotics:

No. of The Bacterial Isolate	Bacterial Isolate	Plasmid Positive	Plasmid Negative
1.	*E. coli*	+	
2.	*E. coli*	+	
3.	*E. coli*	+	
4.	*E. coli*	+	
5.	*E. coli*	+	
6.	*E. coli*	+	
7.	*E. coli*	+	
8.	*E. coli*	+	
9.	*E. coli*	+	
10.	*E. coli*	+	
11.	*E. coli*	+	
12.	*E. coli*	+	
13.	*E. coli*		-
14.	*E. coli*	+	
15.	*E. coli*	+	
16.	*E. coli*	+	
17.	*E. coli*	+	
18.	*Shigella*		-
19.	*Shigella*	+	
20.	*Shigella*		-
21.	*Shigella*	+	
22.	*Shigella*	+	

23.	*Shigella*	+	
24.	*Shigella*	+	
25.	*Shigella*	+	
26.	*Shigella*		-
27.	*Shigella*	+	
28.	*Shigella*		-
29.	*Shigella*	+	
30.	*Shigella*	+	
31.	*Shigella*		-
32.	*Vibrio*	+	
33.	*Vibrio*	+	
34.	*Vibrio*		-
35.	*Vibrio*		-
36.	*Vibrio*	+	
37.	*Vibrio*	+	
38.	*Vibrio*		-
39.	*Vibrio*		-
40.	*Vibrio*		-
41.	*Vibrio*	+	
42.	*Vibrio*		-
43.	*Vibrio*		-
44.	*Vibrio*		-
45.	*Vibrio*	+	

Table 2: Plasmid profiling of 15 isolates showing less resistance to antibiotics:

No. of The Bacterial Isolate	Bacterial Isolate	Plasmid Positive	Plasmid Negative
46.	*E. coli*		-
47.	*E. coli*	+	
48.	*E. coli*	+	

49.	*E. coli*	+	
50.	*E. coli*		-
51.	*Shigella*		-
52.	*Shigella*	+	
53.	*Shigella*		-
54.	*Shigella*		-
55.	*Vibrio*		-
56.	*Vibrio*		-
57.	*Vibrio*		-
58.	*Vibrio*		-
59.	*Vibrio*		-
60.	*Vibrio*		-

From the above result of plasmid profiling the total graph is given below:

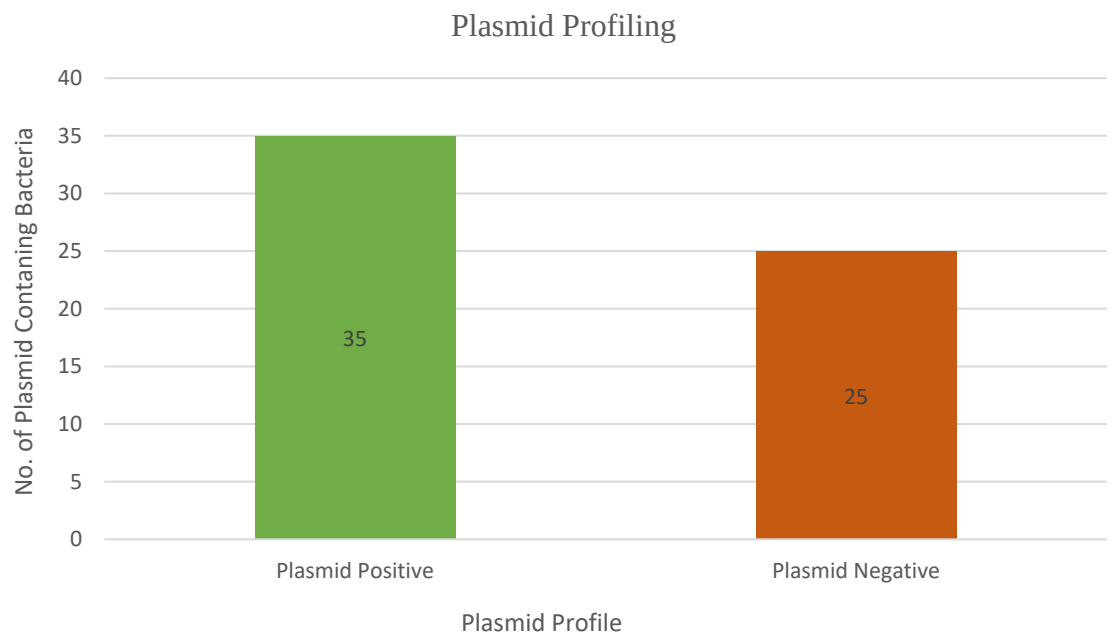

Figure 3.2: Plasmid profiling

From the total result of plasmid profiling of the 60 bacterial isolates, 35 of them resulted plasmid positive and 25 of them resulted plasmid negative.

3.4 Result of Higher Resistant and Lower Resistant Plasmid Profiling:

Here,

Category A= The bacterial isolates which were resistant to all of the antibiotics (*E. coli* -according to the antibiogram result)

Category B= The bacterial isolates which were resistant to most of the antibiotics (*Shigella* -according to the antibiogram result)

Category C= The bacterial isolates which were least resistant to the antibiotics (*Vibrio* -according to the antibiogram result

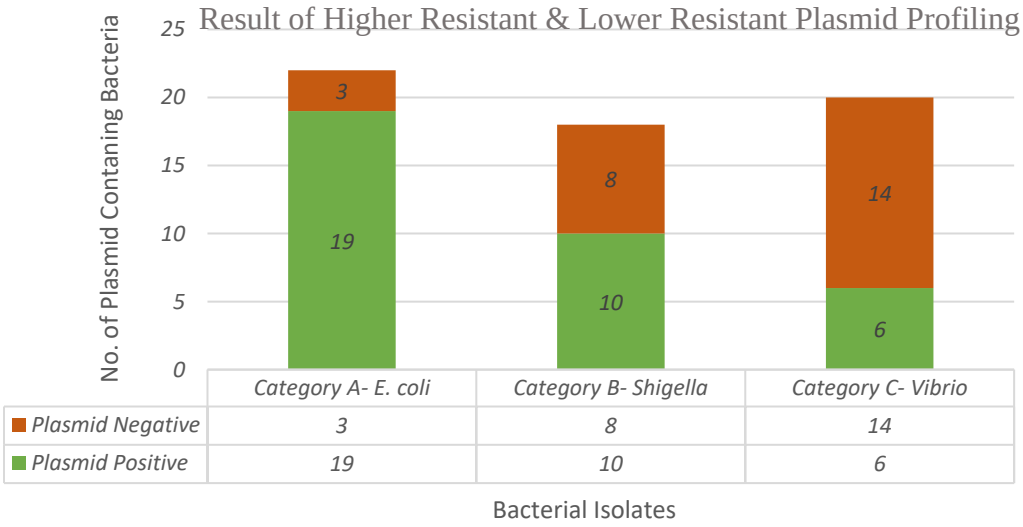

Figure 3.3: Result of Higher Resistant & Lower Resistant Plasmid Profiling

From the 60 bacterial isolates, (Category A) which showed resistance to all of the antibiotics in antibiogram (*E.coli*) are mostly plasmid positive. 19 of them are plasmid positives and 3 of the isolates are plasmid negative. Besides, from the bacterial isolates of (Category B) which were resistant to most of the antibiotics (*Shigella),* 10 of them are plasmid positive and 8 of them are plasmid negative. Moving on, from (Category C) the bacterial isolates which were least resistant to the antibiotics (*Vibrio*), 6 of them are plasmid positive and 14 of them are plasmid negative. Thus, it demonstrates that the bacterial isolates which showed greater resistance in antibiogram are mostly plasmid positive. On the contrary, the bacterial isolates which showed less resistance in antibiogram are mostly plasmid negative.

3.5 Infection Result:

The result of the infection of 2 hours, 6 hours and overnight is shown in a graph below:

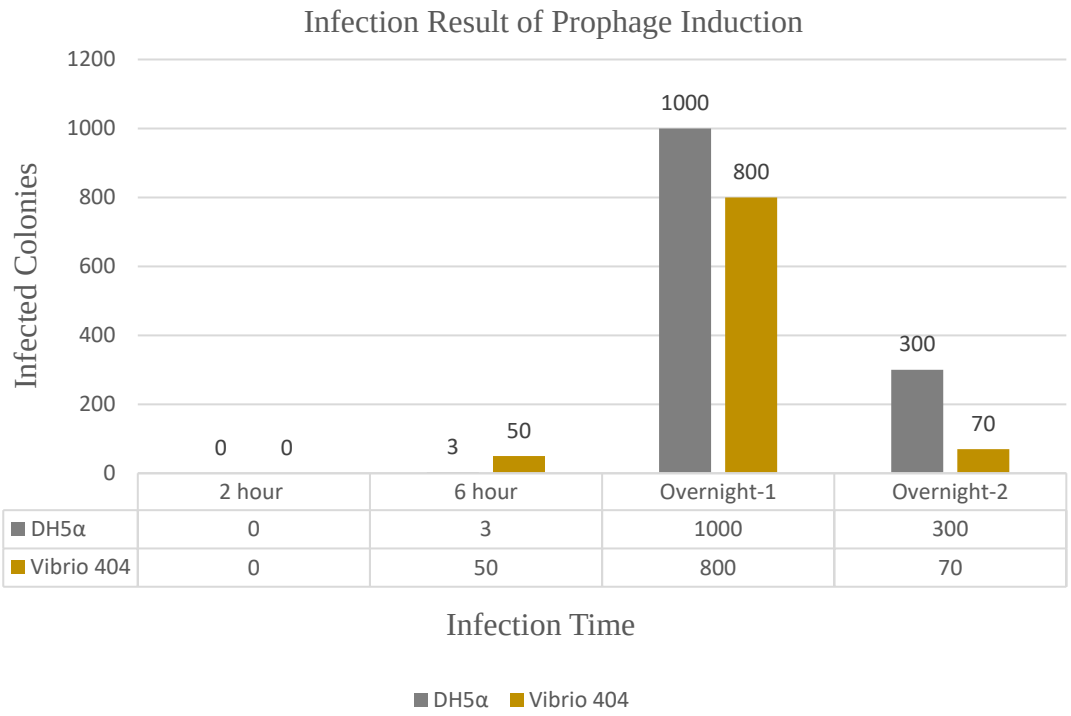

Figure 3.4: Infection Result of Prophage Induction

Here, firstly infection is done for 2 hours. After infection for 2 hours of the prepared supernatant of the bacteria (*E. coli, Shigella, V. cholerae*) 300 µl with the prepared competent cell of DH5α (100 µl) and Vibrio 404 (100 µl) resulted in no growth of colonies in Ampicillin plates (Spread plate technique).

After that, infection for 6 hours of the prepared supernatant of the bacteria (*E. coli, Shigella, V. cholerae*) 300 µl with the prepared competent cell of DH5α (100 µl) and Vibrio 404 (100 µl) resulted in 3 and 50 growths of colonies (resistant) in Ampicillin plates respectively (Spread plate technique).

Then, infection for overnight (1st attempt) of the prepared supernatant of the bacteria (*E. coli, Shigella, V. cholerae*) 300 µl with the prepared competent cell of DH5α (100 µl) and Vibrio 404 (100 µl) resulted in 1000 and 800 growths of colonies (resistant) in Ampicillin plates respectively (Spread plate technique).

Lastly, infection for overnight (2nd attempt) of the prepared supernatant of the bacteria (*E. coli, Shigella, V. cholerae*) 300 µl with the prepared competent cell of DH5α (100 µl) and Vibrio 404 (100 µl) resulted in 300 and 70 growths of colonies (resistant) in Ampicillin plates respectively (Spread plate technique).

3.6 Confirmation by Subsequent Culture:

Table 3: Result of subsequent plate culture

Plate Culture	Positive	Negative
6 hours	+	
Overnight-1	+	
Overnight-2		-

From the infection result of the different time periods (6-hours, Overnight-1, Overnight-2), single resistant colony was subsequently cultured in MacConkey Agar- Ampicillin plate and in Luria Agar- Ampicillin plate. Here, for the subsequent cultures, the resistant colonies from the overnight infection (2nd attempt) did not grow into further colonies in the subsequent culture. Whereas, the resistant colonies from 6-hours infection and overnight infection (1st attempt) grew into successful colonies in the subsequent culture.

3.7 Plasmid Extraction of Infected Colonies:

Table 4: Result of plasmid profiling of the infected colonies

Infected Colonies	Plasmid Positive	Plasmid Negative
6-hour infection- Vibrio 404		-
6-hour infection- DH5α		-
6-hour infection- Vibrio 404		-
Overnight infection- DH5α		-
Overnight infection- Vibrio 404		-

Here, the infected colonies were checked if the plasmid was transferred by plasmid extraction. In result, there were no clear band of plasmid.

CHAPTER 4

DISCUSSION

In this study, the plasmid or the prophage from the environmental bacteria samples can be brought out through UV induction which forms into new viral particles containing the genetic material of its bacteria. This newly formed viral particles can infect with competent cell which in result can grow into successful bacterial colonies by transferring gene harboring the same antibiogram as its corresponding bacteria. Thus, it results into a unique way of gene transfer than the traditional horizontal gene transfer.

The extracted plasmids of the bacteria (*E. coli*, *Shigella* and *V. cholerae)* were resolved in agarose gel electrophoresis. To know the plasmid size, the molecular weight size of unknown plasmids is determined by comparing their band pattern obtained in agarose gel electrophoresis with those obtained with plasmids that have been used as molecular weight or size standards.[20] Here, 1 kb DNA ladder was used which is a unique combination of a number of proprietary plasmids digested with appropriate restriction enzymes to yield 13 fragments, suitable for use as molecular weight standards for electrophoresis. For this ladder, the enhanced intensity of the 1 kb and the 3 kb fragments was designed to ensure quick and easy determination of electrophoresis results.[21] In this research, the extracted plasmids gave result at the enhanced 1kb fragment or in the 1,000 bp size. The extracted plasmid was uncut to run in gel electrophoresis, therefore, it gave two forms of plasmid in its lane. The upper one is the open circular (OC) form and the lower on is the covalently closed circular (CCC) form of plasmid. Relative to the supercoiled covalently closed circular monomer, open circular (OC) moves slower. Compared to the covalently closed circular form, they have more difficulty passing through the pores of the gel matrix. Thus, open circular forms will appear higher in the gel. The supercoiled covalently closed circular monomer, which migrates the fastest, is typically followed by the open circular form.[22]

Prophages are common in the genomes of both clinical[23] and environmental [24] C. difficile isolates. Here, the environmental samples from lake water of Dhanmondi area located at Dhaka contained bacteria (*E. coli*, *Shigella* and *V. cholerae)* which had plasmid as their genetic element. Therefore, this environmental isolate contains prophage integrated into their plasmid. The phage genomes are carried by lysogenic bacteria as "prophage," which are integrated into their own genomes. As a result, the so-called "lysogen" is able to transmit to its progeny the ability to produce phage.[25] To bring out the prophage from bacterial cell, the bacteria cell was induced in UV (ultra-violet transilluminator). DNA-damaging agents (like UV radiation and chemicals) will trigger most

prophages in a population to re-activate. It will help to bring out the prophages as viral particles, which will carry the prophage as its genetic element.

For the growth of the new virus particles after UV induction, the bacteria went for (5-6) hours culture at 37 ºC which will help for the growth of the new virus particles. The bacterial liquid culture containing the new viral particles was centrifuged to purify virus particles. The supernatant contained new viral particles which were filtered with 0.22 µm filter. The competent cell was prepared with 10% glycerol. The bacterial cells (DH5α Cells & Vibrio 404) were grown overnight and harvested because than other stages of growth of cells, cells growing exponentially can be rendered competent more easily. The wash with 10% glycerol helps to make the bacterial cells competent and prepare the cells to accept plasmid DNA.

The freshly prepared competent cell of DH5α Cells & Vibrio 404 then infected with the prepared supernatant. For the infection of 24 hours, the new virus particle containing the bacterial (*E. coli*, *Shigella* and *V. cholerae)* plasmid infected with the prepared competent cell grew into resistant colonies in ampicillin plate. Here for the infection, the virus infects its nucleic acid to the competent cell and then starts to propagate during the infection period. As, the DH5α cells & Vibrio 404 competent cells are sensitive for ampicillin, but after the infection with new viral particles they became resistant. Hence it shows that it is another way of gene transfer not the traditional horizontal gene transfer. Plasmids are important (Horizontal Gene Transfer) HGT (Horizontal Gene Transfer) drivers and can transfer at allegedly high rates by a number of methods, mostly conjugation (including plasmid mobilization and conduction), but also through transduction, transformation, and vesiduction.[26] Moreover, it is producing proof of another way of gene transfer through virus. Several other trials were run for the infection of the new viral particles with the prepared competent cell, for the infection period of 2 hours, there was no growth of colonies observed in the ampicillin plates. The probable reason for this would be the viral particles after infecting its nucleic acid into the competent cell did not have enough time to propagate thus it did not result into growth of resistant colonies in the ampicillin plate. Whereas, for the infection period of overnight and 6 hours respectively resulted into growth of resistant colonies in the ampicillin plates for both of the competent cells (DH5α Cells & Vibrio 404). Which get in the assumption that for the infection result into resistant colonies varies with infection time. In addition to it, the raw sample plating of the infection sample resulted in a greater number of resistant growth

of colonies than the 10-1 diluted infected sample plating. Then, for the confirmation of this growth subsequent culture was done. For the growth into DH5α competent cells, the resulted resistant single colonies were cultured into MacConkey Agar ampicillin plate. It was observed that, from the overnight infection result, there was no growth of colonies in the subsequent cultures whereas, the 6-hour infection result gave resistant growth of colonies in the MacConkey Agar ampicillin plate. Likewise, for the growth into Vibrio 404 competent cells, the resulted resistant single colonies were cultured into Luria Agar ampicillin plate. The observation was same as like DH5α competent cells. There was no growth of resistant colonies for the overnight infection result but resulted into resistant growth of colonies for the 6 hours infection result in the subsequent cultures. From this, it assumes that the resulted colonies from the overnight infection could not withstand in the subsequent culture even though it resulted into greater number of colonies comparing to the resulted colonies of 6 hours infection.

Here, the objective of this research is to see by prophage inducing it is working in gene transfer or not. I wanted to see in the infected particles, the plasmid is transferring or the plasmid is being packaged or not, for that plasmid extraction was done. In the gel electrophoresis of plasmid extraction, it did not result into any clear plasmid bands in the agarose gel for several trials. As the bacterial isolates contained plasmid, so it was assumed that probably the plasmid gene can be transferred to new viral particles by this prophage induction. The prophage can carry plasmid to the newly formed viral particles as circular manner integrated into the plasmid or by integrating into the chromosomal DNA of the newly formed viral particles. In this study, the aim was to observe if the plasmid is being packaged to the newly formed viral particles or not and as it did not result into clear plasmid bands in the gel electrophoresis of plasmid isolation for the infected particles, the speculation would be that the prophage is carrying the gene to transfer to the newly formed viral particles but they are integrated into the chromosomal DNA. Hence, it can be observed as, the gene is getting transferred of these bacteria thus I have got transformed newly formed colonies after the infection.

Conclusion

Allover, this study demonstrating that, apart from the traditional approach of transferring gene, infection between new viral genes containing plasmid or prophage as its genetic element with bacterial competent cell can result in transfer of antibiotic resistance ability. Till now, little is known about gene transfer through infection than HGT. Along with it, some other approaches have also been reported for gene transfer which is not many comparing to HGT. Antibiotic resistance genes (ARGs) are expected to spread horizontally through biofilms in aquatic environments. Although methods are understood and have been demonstrated to depend on the environment, bacterial populations, and mobile genetic elements, ARGs can spread via HGT. Traditional interspecies HGT processes include conjugation, transformation, and transduction; more recently, membrane vesicles (MVs) have been described as potential DNA reservoirs.[27] Likewise, in this study, gene transferring via infection is a unique approach to the knowledge of transferring gene and requires further study and analysis to get going.

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