Bacteriophage mediated Horizontal Gene Transfer

By

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of Bachelor of Science in Microbiology

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing Bachelor of Science in Microbiology 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 have acknowledged all main sources of help.

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Approval

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Of fall 2021 semester has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Microbiology on 29 November, 2021.

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Ethics Statement

This material is an original work, which has not been previously published elsewhere. It is my own research and analysis in a truthful and complete manner. The paper properly credits all the sources used (correct citation).

Abstract

Bacteriophages are viruses which infect and replicates within the bacterial cell. Bacteriophage refers ‘bacteria eater’ that means the bacteriophages kill their host cell because bacteriophage is a bacteria-infecting virus. By using this mechanism and also associated with Horizontal Gene Transfer mechanism, bacteriophages transfer various types genetic material in to the bacterial cells. Horizontal Gene Transfer (HGT) occurs through three well-understood genetic mechanisms which are transformation, conjugation and transduction.

This review paper contains bacteriophages horizontally transfer various types of genetic materials such as antibiotic resistant genes (ARGs), virulent genes, and multidrug resistant genetic materials and also the bacteriophage can be used as therapeutic agent which can be referred as phage therapy. Bacteriophages convert antibiotic susceptible bacteria to antibiotic resistant bacteria through horizontal gene transfer following generalized or specialized transduction. Using broad spectrum antibiotics activate endogenous prophages in multidrug resistant bacteria and as a result, those prophages produce infectious virions. As a result, phage therapy can be used as an alternative to antibiotics to solve this problem. The phage virus is highly specific. This indicates that the phage virus will only infect the targeted bacterial pathogen and would not harm the surroundings. Because of the bacteriophage-mediated virulent genetic transfer to bacterial strains, Bacteriophages are responsible for transforming a non-pathogenic bacterial strain into a pathogenic one.

This work is dedicated to

My Beloved Parents

Acknowledgement

I am grateful to Professor Dr. A F M Yusuf Haider (Chairperson Department of Mathematics and Natural Sciences, BRAC University) for looking after all of the students and teachers in his department and for always being willing to lend a helping hand when needed.

I owe a great debt of gratitude to Dr. Iftekhar Bin Naser sir for guiding me throughout my thesis project and providing all of the assistance which i required. Sharing his enormous experience and knowledge with me was really beneficial to my project.

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Sincerely,

ARPON KUMAR DAS

Department of Mathematics and Natural Sciences, BRAC University

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List of Acronyms

STEC- Shiga Toxin Producing *E. coli*

STX- Shiga Toxin

HGT- Horizontal Gene Transfer

ARGs- Antibiotic Resistant Genes

MGEs- Mobile Genetic Elements

WWTP- Waste Water Treatment Plant

MAR- Multiple Antibiotic Resistant

GTAs- Gene Transfer Agents

VAPGHs- Virion Associated Peptidoglycan Hydrolases

LPS- Lipopolysaccharide

PGs- Peptidoglycans

VT- Verotoxin

VTEC- Verotoxin Producing *E. coli*

MDR- Multidrug Resistant

PCR- Polymerase Chain Reaction

OD- Optical Density

1. Introduction

A bacteriophage is a bacteria-infecting virus. Because bacteriophages kill their host cells, the word "bacteriophage" literally means "bacteria eater." A nucleic acid molecule is enveloped by a protein structure in all bacteriophages. The nucleic acid can be double-stranded or single-stranded, and it can be DNA or RNA. The name "bacteriophage" comes from two words: "bacteria" and "phagein," which all mean "to devour. Félix d'Hérelle coined the phrase. Their genomes may encode as little as four genes (for example, MS2) or as many as hundreds. Following the injection of their DNA into the bacterium's cytoplasm, phages proliferate within it. Phages are classified into three basic structural forms: an icosahedral (20-sided) head with a tail, an icosahedral head without a tail, and a filamentous form.

Horizontal gene transfer (HGT) is the movement of genetic information between organisms, which includes the spread of antibiotic resistance genes among bacteria (except those from parent to offspring), fueling pathogen evolution. Many resistance genes evolved long ago in natural environments with no anthropogenic influence, but these genes are now rapidly spreading to and among human pathogens. HGT occurs through three well-understood genetic mechanisms which are transformation, conjugation and transduction. In case of transformation, bacteria take up DNA from their environment. The term conjugation refers that bacteria directly transfer genes to another cell and transduction indicates that the bacteriophages (bacterial viruses) move genes from one cell to another. Once transferred, the genes and pathogens continue to evolve, frequently resulting in more resistant bacteria. Natural selection can cause all genes, not just those that cause drug resistance, to be horizontally transferred and spread, including virulence determinants.

My prior work revealed that bacteriophages horizontally transfer various types of genetic materials such as antibiotic resistant genes (ARGs), virulent genes, and multidrug resistant genetic materials and also the bacteriophage can be used as therapeutic agent which can be referred as phage therapy. Bacteriophages convert antibiotic susceptible bacteria to antibiotic resistant bacteria through horizontal gene transfer following generalized or specialized transduction. ARGs may be acquired and transferred among bacteria via mobile genetic elements (MGEs) such as conjugative plasmids, insertion sequences, integrons, transposons, and phages. However, new research suggests that phages may play a larger role in the emergence and spread of ARGs than previously thought. Antibiotics may have unforeseen implications in human and veterinary medicine. As a result, antibiotic exposure can lead to antibiotic resistance; antibiotic-associated diarrhea, increased pathogen invasion, and stimulation of horizontal gene transfer. Fluoroquinolones are a type of antibiotic that is used to treat a wide range of infectious diseases in both humans and animals. Fluoroquinolone exposure activates endogenous prophage in S. Typhimurium DT104 and DT120 via up regulating the bacterial recA gene, which activates the bacterial SOS response. This SOS response not only stimulates bacterial DNA repair that has been damaged by fluoroquinolone, but it also activates prophage, which produces infectious

virions. Because of the overuse of antibiotics, multidrug resistant bacteria are now prevalent in our environment. As a result, phage therapy can be used as an alternative to antibiotics to solve this problem. So, here has been focused on that how phage therapy can be used as an alternative to antibiotics. Bacteriophages are responsible for transforming a non-pathogenic bacterial strain into a pathogenic one. This is due to bacteriophage-mediated virulent genetic transfer to bacterial strains. As a result, bacteriophages are known as converting phages or bacteriophages. Temperate bacteriophages have always played an important role in bacterial evolution. Through the spread of virulence and antibiotic resistance genes, bacteriophages' transductive and lysogenic abilities have contributed to the evolution and shaping of emerging foodborne pathogenic bacteria. The basic genetic exchange mechanisms mediated by temperate bacteriophages are described here, as well as how these mechanisms have been pivotal in the spread of virulence genes, such as toxins and antibiotics, from one species to another. Temperate bacteriophages contribute to the ongoing evolution of pathogenic bacteria.

2. Life Cycles of Bacteriophage

Bacteriophages reproduce in the bacterial host cell, just as all viruses. Viral lifecycles are divided into two types, each with its own DNA replication pathway. The viral DNA replicates independently of the host DNA (Lytic Cycle) in one and is introduced into the host DNA in the other (Lysogenic Cycle). These lifecycles can occur simultaneously or alternately in diverse types of bacteriophages.

2.1 The Lytic Cycle

The bacteriophage takes over the cell, reproduces additional phages, and then destroys the cell during the lytic cycle of virulent phage. T-even phage is a well-known virulent phage class. In the bacteriophage lytic cycle, there are five phases (see Figure 1). The phage interacts with certain bacterial surface receptors during attachment, the first stage of the infection process. Entry, or penetration, is the second stage of infection. The viral genome is injected into the cell wall and membrane by the contraction of the tail sheath, which works like a hypodermic needle.

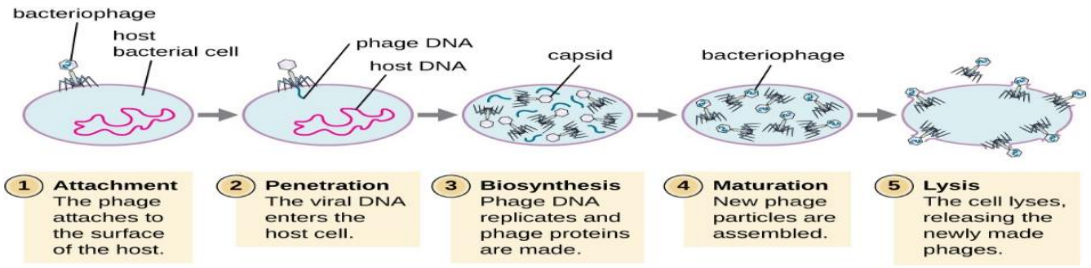

Figure 2.1: Lytic Cycle of Bacteriophage

The manufacture of new viral components is the third stage of infection. Maturation is the fourth stage of infection; where new phage particles are assembled. Lysis is the fifth stage of infection..

2.2 The Lysogenic Cycle

Lysogeny, or the lysogenic cycle, is one of two cycles of viral reproduction (the lytic cycle being the other). Lysogeny is characterized by integration of the bacteriophage nucleic acid into the host bacterium's genome or formation of a circular replicon in the bacterial cytoplasm.

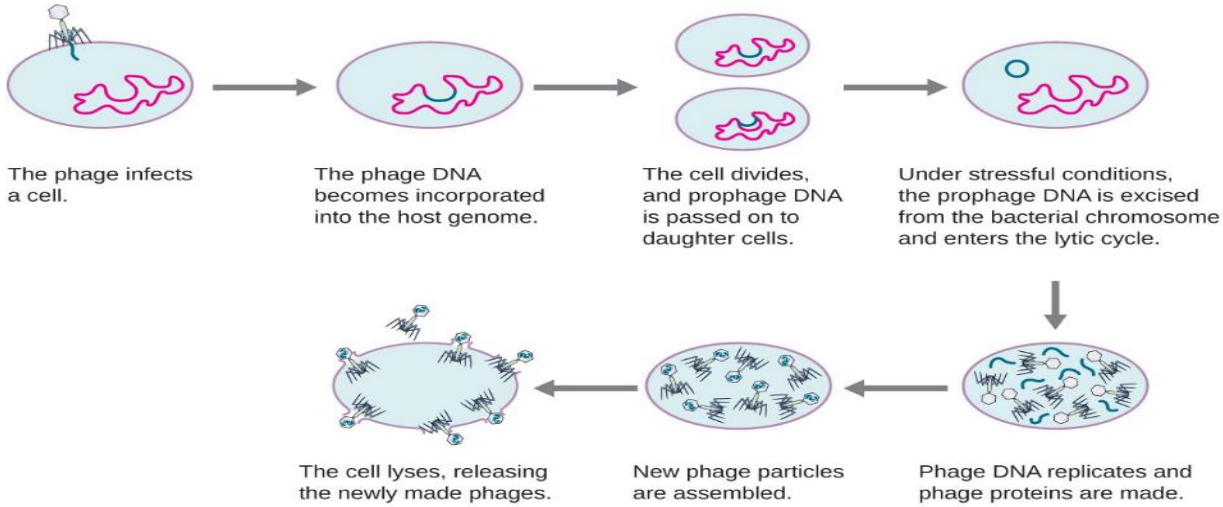

Figure 2.2: Lysogenic Cycle of Bacteriophage

3. Horizontal Gene Transfer (HGT)

Horizontal gene transfer (HGT) refers to the movement of genetic material across unicellular and/or multicellular organisms that are not accomplished through the ("vertical") transmission of DNA from parent to offspring (reproduction). Many creatures have evolved as a result of HGT. Horizontal gene transfer is the most common way for bacteria to propagate antibiotic resistance, and it's also critical for the evolution of bacteria that can digest new substances like pesticides developed by humans, as well as the evolution, maintenance, and transmission of virulence.

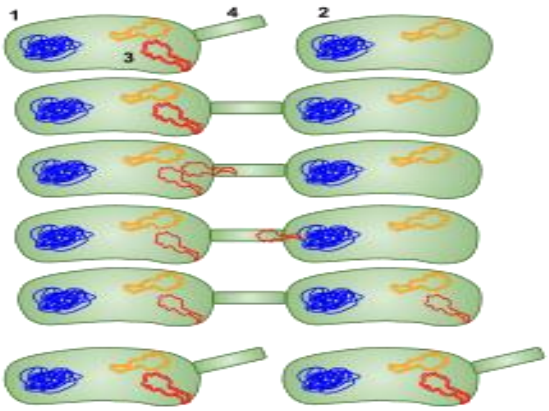

Figure 3: Horizontal Gene Transfer

3.1 Different way of Horizontal Gene Transfer (HGT)

In bacteria, horizontal gene transfer occurs through three mechanisms: transformation, transduction, and conjugation. Conjugation is the most common process for horizontal gene transmission across bacteria, especially from one donor bacterial species to another. Although bacteria can acquire new genes through transformation and transduction, this is a relatively rare occurrence among bacteria of the same or closely related species.

3.1.1 Transformation:

Transformation is a type of genetic recombination in which a DNA fragment from a degraded, dead bacterium is introduced into a competent recipient bacterium and exchanged for a bit of the recipient's DNA. Homologous recombination, or the recombination of homologous DNA sections with roughly identical nucleotide sequences, is frequently all that is required for transformation. Similar bacterial strains or strains from the same bacterial species are most commonly involved in this.

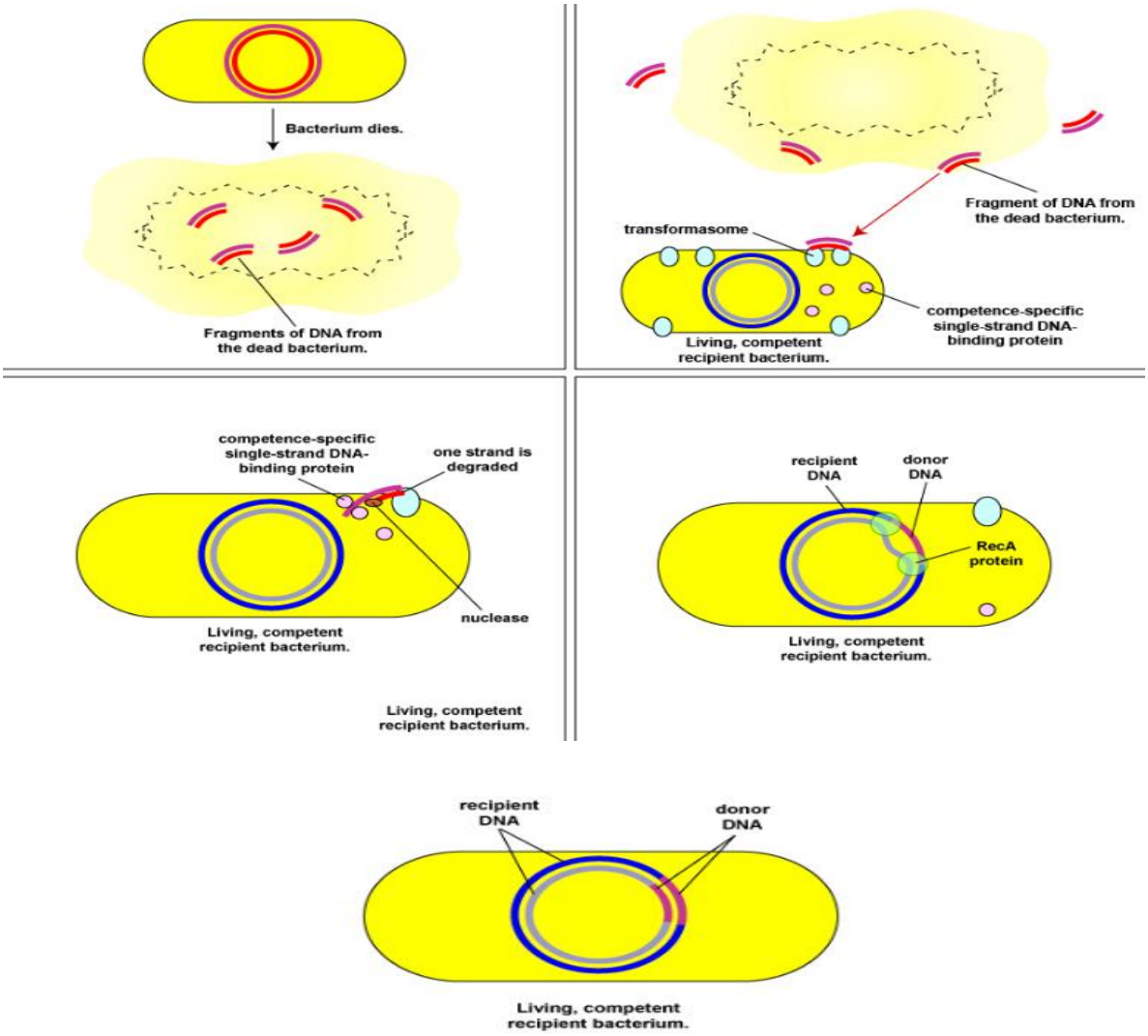

Figure 3.1.1: Transformation: Step 1: A donor bacterium dies and is degraded. Step 2: DNA fragments, typically around 10 genes long, from the dead donor bacterium bind to transformasomes on the cell wall of a competent, living recipient bacterium. Step 3: In this example, a nuclease degrades one strand of the donor fragment and the remaining DNA strand enters the recipient. Competence-specific single-stranded DNA-binding proteins bind to the donor DNA strand to prevent it from being degraded in the cytoplasm. Step 4: RecA proteins promotes genetic

exchange between a fragment of the donor's DNA and the recipient's DNA. This involves breakage and reunion of paired DNA segments. Step 5: Transformation is complete.

3.1.2 Transduction:

The transfer of a DNA fragment from one bacterium to another by a bacteriophage is known as transduction. Generalized transduction and specialized transduction are the two types of transduction.

Occasionally, the phage capsid accidentally assembles around a small portion of bacterial DNA during the replication of lytic and temperate bacteriophages. When this bacteriophage, known as a transducing particle, infects another bacterium, it injects the donor bacterial DNA fragment into the recipient, where it can be exchanged for a piece of the recipient's DNA through homologous recombination.

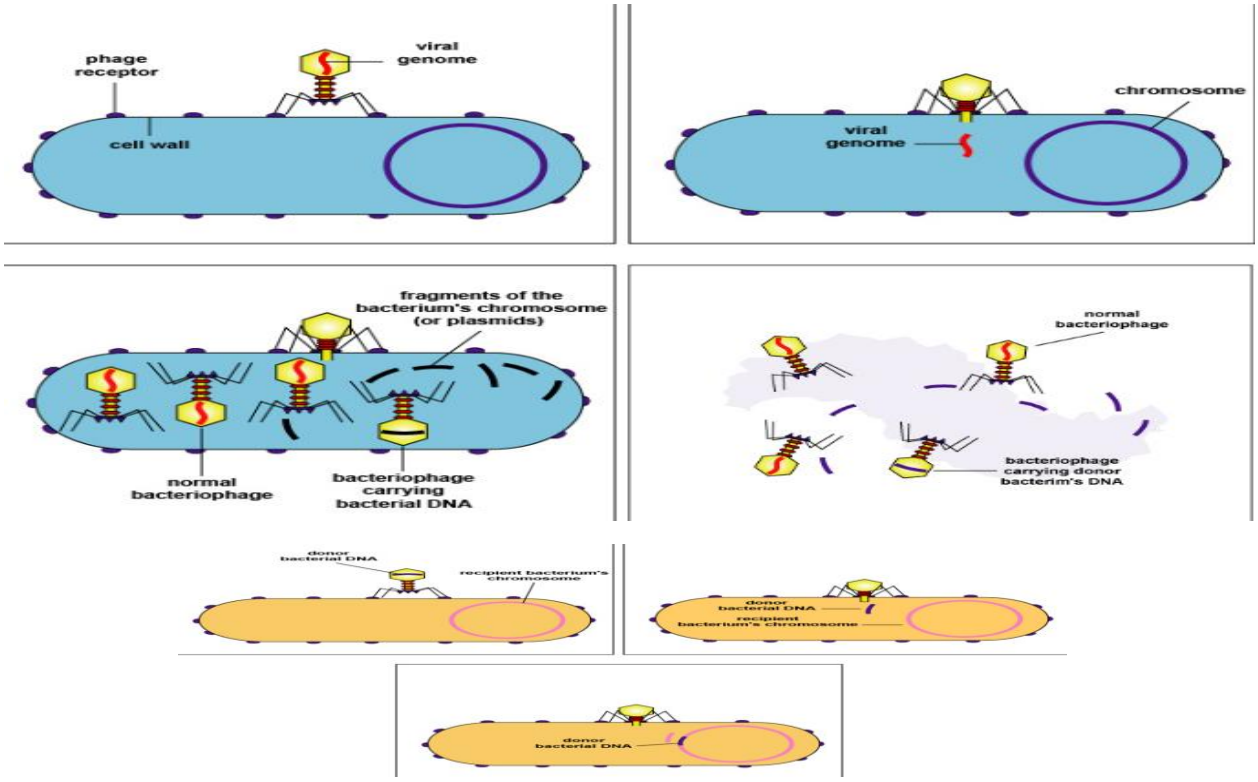

Figure 3.1.2: Generalized Transduction.

3.1.3 Conjugation:

Bacterial conjugation is the transfer of genetic material between bacterial cells by direct cell-to-cell contact or a bridge-like link. This is accomplished through the use of a pilus. In bacteria, it is a parasexual way of reproduction. It, like transformation and transduction, is a horizontal gene transfer method; however these two do not involve cell-to-cell contact.

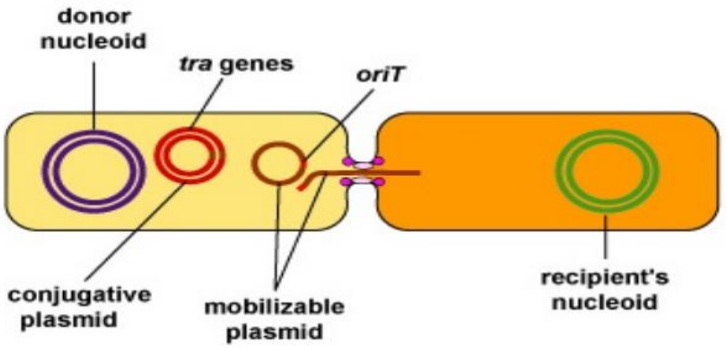

Figure 3.1.3: Transfer of Mobilizable Plasmids during Conjugation. Mobilizable plasmids, that lack the tra genes for self-transmissibility but possess the oriT sequences for initiation of DNA transfer, may also be transferred by conjugation if the bacterium containing them also possesses a conjugative plasmid. The tra genes of the conjugative plasmid enable a mating pair to form while the oriT sequences of the mobilizable plasmid enables the DNA to move through the conjugative bridge.

Plasmids and transposons code for conjugation. It involves a conjugative plasmid-containing donor bacteria and a non-conjugative plasmid-containing recipient cell. A conjugative plasmid is self-transmissible because it contains all of the required genes for it to conjugate with another bacterium. Tra genes enable the bacteria to form a mating pair with another organism, whereas oriT (origin of transfer) sequences define where on the plasmid DNA transfer begins by serving as the replication start site where DNA replication enzymes nick the DNA to begin replication and transfer.

4. Bacteriophage as Transporters of Antibiotic Resistance Genes in the environment

This article will, therefore, describe the current knowledge on the emergence and spread of antibiotic resistance in the environment, with special emphasis on the role of phages in the mobilization of antibiotic resistant genes (ARGs). Antibiotic resistance is spreading through the widespread use of antibiotics has contributed to the increase of antibiotic resistant bacteria, including those causing infections in both humans and animals. In terms of mobile genetic elements (MGEs), Bacteriophages convert antibiotic susceptible bacteria to antibiotic resistant bacteria through horizontal gene transfer following generalized or specialized transduction. Understanding sources and mechanisms of antibiotic resistance is critical for developing effective strategies for reducing their impact on public and environmental health.

The large-scale mixing of environmental bacteria with exogenous bacteria from anthropogenic sources provides the ideal selective and ecological conditions for the emergence of resistant bacteria as well as the environment is continually exposed to a wide variety of antimicrobials and their metabolites through wastewater treatment plant (WWTP) discharges, agricultural runoff, and animal feeding operations, as a result of emergence and spread of Antibiotic resistant genes (ARGs). ARGs may be acquired and transferred among bacteria via mobile genetic elements (MGEs) such as conjugative plasmids, insertion sequences, integrons, transposons, and phages. Recent findings, however, suggest that phages may play a more significant role in the emergence and spread of ARGs than previously expected.

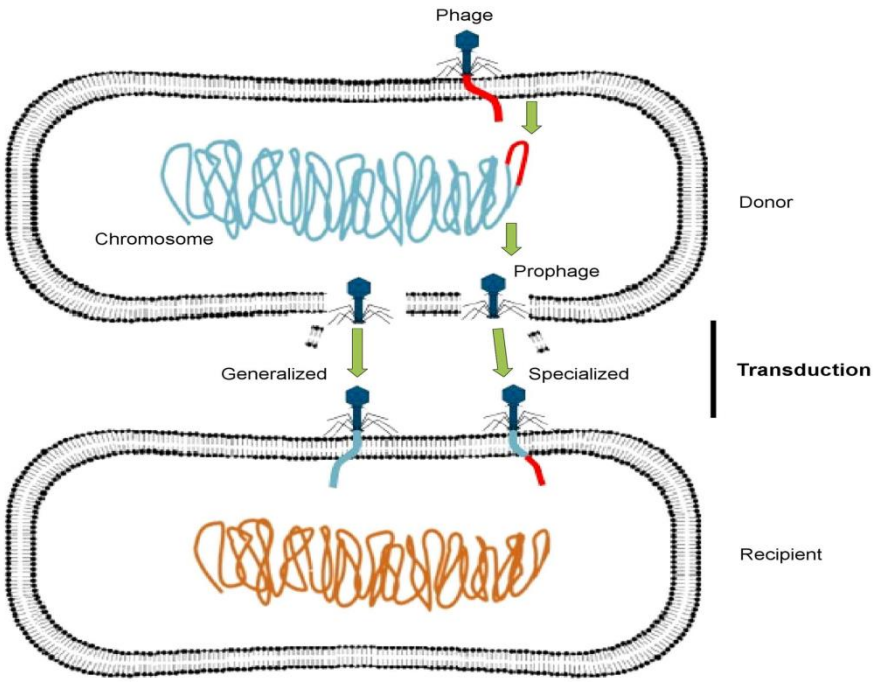

Figure 4.1: Transfer of DNA between bacteria via phages. A temperate phage inserts its genome (red) into the bacterial chromosome (bluegreen) as a prophage, which replicates along with the bacterial chromosome, packaging

host DNA alone (generalized transduction) or with its own DNA (specialized transduction). It then lyses the bacterial cell, releasing progeny phage particles into the surrounding environment. After lysis, these phages infect new bacterial cells, in which the acquired DNA recombines with the recipient cell chromosome (orange). This figure has been adapted from Frost et al. [2].

A study using real-time PCR (qPCR) assays revealed the presence of two genes (blaTEM and blaCTX-M) encoding b-lactamases and one gene (mecA) encoding a penicillin-binding protein in phage DNA from urban sewage and river water samples. In contrast to the previous study, the authors demonstrated that those ARGs (blaTEM and blaCTX-M) from phage DNA were transferred to susceptible E. coli strains, which became resistant to ampicillin. Another study of phage DNA from different hospital and urban treated effluents using qPCR assays showed the presence of high levels of genes (blaTEM, blaCTX-M and blaSHV) conferring resistance to b-lactam antibiotics, as well as genes (qnrA, qnrB and qnrS) conferring reduced susceptibility to fluoroquinolones. A recent study demonstrated the presence of the qnrA and qnrS genes in phage DNA from fecally polluted waters and the influence of phage-inducing factors on the abundance of those ARGs. They observed that urban wastewater samples treated with chelating agents, such as EDTA and sodium citrate, showed a significant increase in the copy number of those ARGs in phage DNA compared to the nontreated samples. So, these studies not only suggest that anthropogenic inputs may facilitate the emergence of ARGs but also demonstrate the contribution of phages to the spread of ARGs into the environment.

Metagenomic Exploration of Antibiotic Resistance in Environmental Settings

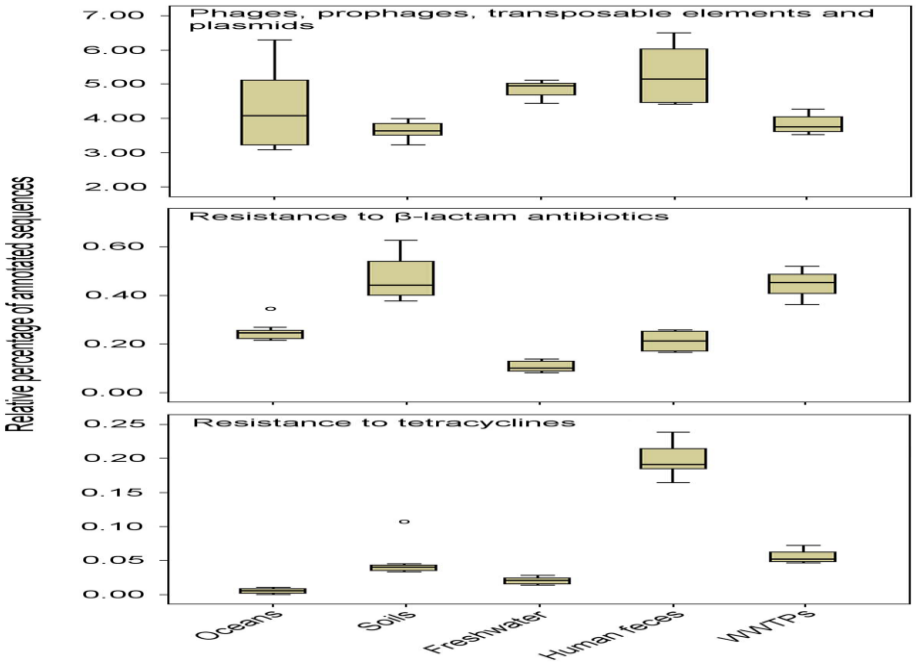

Figure 4.2. Metagenomic exploration of the resistome from human and environmental sources. Relative distribution of reads assigned to three functional subsystems among 27 metagenomes (based on MG-RAST annotation, E-value = 1025). Data are normalized by the total annotated sequences and are expressed as a percentage. The horizontal line in each box plot represents the mean of the relative distribution in each of the five environments (oceans, soils, freshwater, human feces, and WWTPs). Accession numbers for oceans: 4441573.3, 4441574.3, 4441576.3,

4441577.3, 4441591.3, and 4443729.3; soils: 4441091.3, 4445990.3, 4445993.3, 4445994.3, 4445996.3, and 4446153.3; freshwater (rivers): 4511251.3, 4511252.3, 4511254.3, 4511255.3, 4511256.3, and 4511257.3; human feces: 4440595.4, 4440460.5, 4440611.3, 4440614.3, 4440825.3, and 4461119.3; and WWTPs: 4455295.3, 4463936.3, and 4467420.3.

A comparison of 27 metagenomes (data available publically) corresponding to several projects and environments using the MG-RAST platform revealed a relatively high proportion of sequences related to MGEs, including phages, among microbial communities from different natural environments . The comparative analysis also revealed that the sequences related to genes conferring resistance to b-lactam antibiotics were detected more frequently among microbial communities from soil and WWTP environments than those from oceans, freshwaters, and human feces. Likely, the sequences related to genes encoding tetracycline resistance were more abundant among microbial communities from human feces. These results suggest that natural environments are a substantial source of mobile genetic elements (MGEs), which may contribute to the horizontal transfer and spread of ARGs.

Antibiotic resistant bacteria are available and increasing day by day. The mobile genetic elements like phages, plasmids, transposons are playing important role for spreading antibiotic resistant bacteria. It is now clear that the environment is a vast reservoir of resistant organisms and their associated genes. ARGs found in human microbial communities have been acquired from environmental sources. Metagenomic approaches offer valuable tools to explore the microbial community composition, ARGs, and MGEs in different environments. The role of mobile genetic elements (MGEs) such as phages as vehicles for antibiotic resistant genes (ARGs) from different environments can be determined by using clone libraries and functional screening. The use of Metagenomic data generated via high-throughput sequencing has revealed that most ARGs found in nonpathogenic soil bacteria have perfect nucleotide identity to ARGs from several human pathogens, which is suggesting that recent horizontal gene transfer via MGEs has occurred between those organisms. Metagenomic studies also demonstrate that phages, which may act as vehicles for ARGs, are widely distributed in nature.

5. Temperate Bacteriophage-Mediated Gene Transfer in the Evolution of Foodborne Pathogens

Temperate bacteriophages have always been central to the evolution of bacteria. The transductive and lysogenic capacities of bacteriophages have contributed to the evolution and shaping of emerging foodborne pathogenic bacteria through the dissemination of virulence and antibiotic resistance genes. Here has been described the basic genetic exchange mechanisms mediated by temperate bacteriophages and how these mechanisms have been central to the dissemination of virulence genes, such as toxins and antibiotics from one species to another. The temperate bacteriophages play a role in the on-going evolution of emerging pathogenic bacteria.

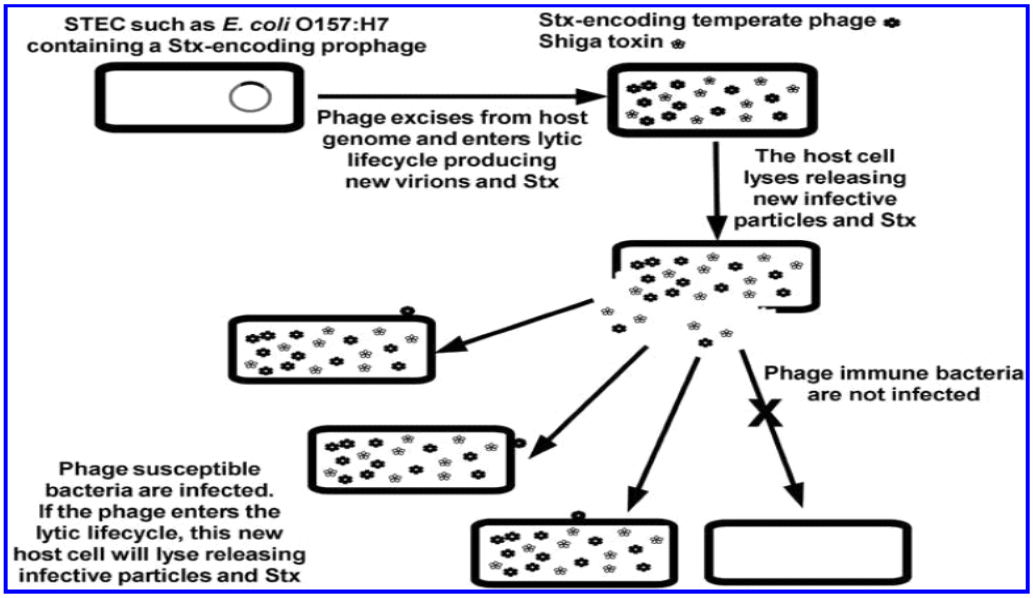

Figure 5.1: stx encoding phage and shiga toxin amplification by native gastrointestinal microflora (Gamage et al., 2003).

Matsushiro et al. (1999) observed a 1000-fold increase in bacteriophages titers and a 60-fold increase of Shiga toxin production within 6 hours of *in vitro* ciprofloxacin exposure. These new infective bacteriophage particles can then potentially lysogenize new bacteria, such as non- STEC *E. coli*, and convert harmless commensal microflora into STEC, further magnifying toxin production. *In vivo* studies of *stx* lysogenic conversion, clearly demonstrated *stx* gene movement across a diverse group of *E. coli* and also

illustrate that induced Stx-encoding bacteriophages can cause an amplification of Stx production by infecting susceptible commensal *E. coli* in the gastrointestinal tract of the diseased individual. The gut flora of diseased individual is susceptible to bacteriophage infection and so acts to amplify toxin production and the healthy gut flora is resistant to bacteriophage infection. The Shiga toxins were first identified in, purified from, which is gastrointestinal pathogen *Shigella dysenteriae*, which is the only *Shigella* strain known to produce Shiga toxins. The stx gene in *S. dysenteriae* is virtually indistinguishable from the stx1 gene in *E. coli* O157:H7. Both *S. dysenteriae* type 1 and STEC (O157:H7 specifically) produce Shiga toxins and carry genes that encode these toxins. *E. coli* and *Shigella* are so closely related. One major difference between the *Shigella* and *E. coli* was the genetic structure of their stx genes; in O157:H7 the stx genes are present within integrated bacteriophages, but in *S. dysenteriae* stx1 is chromosomal. It has been suggested that transduction and lysogenic conversion of *E. coli* by bacteriophages has caused some of the most dynamic changes within this bacterial species, with one of the most interesting being the Shiga toxin-encoding bacteriophages of *Shigella* and *E. coli* O157:H7. Genes encoded in bacteriophage genomes in many instances, be more stable in the environment than those resident in the bacterial genome. For example, *in situ* river water stability experiments have shown that Shiga-like toxin 2 (Stx2)–encoding bacterial titers decrease 2–3 log10 compared to a 1–2 log10 reduction in *Stx2*-encoding bacteriophage titers over the same period. A number of temperate bacteriophages have been isolated from the environment that encodes either *stx1* or *stx2* with a great deal of variability in their sequence, host range, and infection characteristics. Within the enteric *E. coli* strains, temperate bacteriophages continue to transfer the *stx* genes to new hosts and therefore potentially create new types of Shiga toxin–producing *E. coli.* Using a recombinant bacteriophage, James et al. (2001) found that, of 113 bacterial strains tested, 30 *E. coli* (including both commensal and pathogenic species isolated from sheep, cattle, pig, and humans) from diverse serotypes and all four *Shigella* strains were infected and underwent lysogenic conversion, expressing kanamycin resistance (James et al., 2001). A similar study by Schmidt et al. (1999), using a detoxified bacteriophage derivative, found that none of the *E. coli* strains tested supported plaque formation, but a number were infected by the bacteriophage and underwent lysogenic conversion, becoming chloramphenicol resistant (Schmidt et al., 1999). Most recently, Gamage et al. (2004) experimentally infected all 72 members of the *E. coli* collection of reference (ECOR, a collection that was selected to represent the broad genetic diversity of the *E. coli* genus), with *Ø*933W*Δ*stx2 (Gamage et al., 2004). This single bacteriophage was able to lysogenize 43% of the collection (31/72) and, in some instances, induce cell lysis with a concomitant increase in Stx toxin production. Together these studies clearly show that temperate bacteriophages can lysogenize and thus spread the *stx* genes widely across the *E. coli* genus, including strains of *E. coli* that already play host to prophages. The shiga toxin encoding bacteriophages are able to infect and lysogenize a wide range of

bacteria; the Stx-encoding temperate bacteriophage of *Shigella sonnei* is able to transduce chromosomal genes in the laboratory *E. coli* strain K-12. It has also been shown to have a broad host range, including *S. dysenteriae*, *S. flexneri*, *S. boydi*, *S. sonnei*, and *E. coli*, and is able to integrate into the genomes of both non-toxigenic *S. sonnei* and *E. coli* strains via generalized transduction. The recent genomic studies of the pathogens *S. dysenteriae* and *E. coli* O157:H7, along with the Stx-encoding temperate bacteriophages, show the key role bacteriophage mediated gene transfer has played in the evolution of these high-profile human pathogens. The current experimental evidence stated that the versatility and ease with which Stx encoding bacteriophages infect and transduce wild-type and pathogenic strains of *E. coli* demonstrates that these prophages continue to mediate the transfer of virulence genes within the *E. coli* family and beyond, and may be critical in the emergence of new pathogens and continued pathogen evolution. Finally, it must also be noted that bacteriophage-mediated gene transfer rates may be even higher in the mammalian gut than previously thought. Multiple antibiotic resistant (MAR) bacterial strains are available in poultry worldwide. One of the most notable MAR strains is *Salmonella enterica* serovar Typhimurium DT104, which is resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides, tetracyclines (called ACSSuT resistance type) and, more recently, fluoroquinolones, apramycin and trimethoprim, as well as reduced susceptibility to ciprofloxacin. *Salmonella Typhimurium* DT104 is the culprit behind many salmonellosis outbreaks around the world and has been one of the two most common serovars isolated from human salmonellosis cases in the United States. Food animals such as poultry, cattle and swine are the primary reservoirs of *Salmonella* and can harbor antibiotic-resistant strains. Study showed that, two bacteriophages (ES18 and PDT17) have been shown to transduce the antibiotic resistance genes in *S. Typhimurium* DT104, with PDT17 integrated into the genome of all strains of DT104. Antibiotic resistance seen in several common foodborne bacterial strains such as *E. coli*, *Salmonella*, and *P. aeruginosa.*

Virulence factor (gene)	*Example bacteriophage(s)*	*Bacterial host(s)*	*Reference*
Shiga toxin 1 (Stx1)	H-19B, VT2-Sakai	*Escherichia coli* *Shigella dysenteriae* *Shigella* spp.	De Grandis *et al.*, 1987; Makino *et al.*, 1999
Shiga toxin 2 (Stx2)	933W, ϕ3538 VT1-Sakai	*Escherichia coli* *Shigella dysenteriae* *Shigella* spp.	Plunkett *et al.*, 1999; Schmidt *et al.*, 1999; Yokoyama *et al.*, 2000
Cholera toxin (*ctxAB*)	CTXϕ	*Vibrio cholerae* *Shigella dysenteriae* *Shigella* spp.	Faruque *et al.*, 1998; Mekalanos *et al.*, 1997; Waldor *et al.*, 1997
Type III effector protein SopE	SopEϕ	*Salmonella enterica* serovar Typhimurium	Hapfelmeier *et al.*, 2004; Mirold *et al.*, 1999
Cytotoxin	ϕCTX	*Pseudomonas aeruginosa*	Nakayama *et al.*, 1999
Exofoliative toxin A	ϕMU50A, ϕETA	*Streptococcus pyrogenes*	Yamaguchi *et al.*, 2000
Exotoxin A (erythrogenic toxin)	T12	*Streptococcus pyrogenes*	Weeks and Ferretti, 1984
Enterotoxin A Enterotoxin P	ϕ13, ϕ315	*Staphylococcus aureus*	Betley and Mekalanos, 1985; Casman, 1965; Coleman *et al.*, 1989
Botulinum toxin C	c-st, CEβ	*Clostridium botulinum*	Eklund *et al.*, 1971; Tsuzuki *et al.*, 1990
Botulinum toxin D	d16ϕ	*Clostridium botulinum*	Eklund *et al.*, 1972; Sunagawa *et al.*, 1992

A number of other food and waterborne pathogens, such as *Clostridium difficile*, have been shown to contain prophages, but their precise interaction is not known at this time.

Table 5.2: Examples of virulence factors encoded by bacteriophages found in food and waterborne pathogens.

We know that temperate bacteriophages are responsible for evolution of emerging pathogenic bacteria. In this article, here has been emphasized on the food borne pathogenic bacteria and their pathogenic activity enhanced by the temperate bacteriophages. Normally, the infective bacteriophages lysogenize new bacteria and convert the non STEC to STEC. We know that STEC *E.Coli* is a foodborne pathogenic bacteria that cause foodborne disease. Here we have been seen that the stx encoding phage lysogenized the STEC *E.Coli 0157:H7.* After that, the prophage has been excised from host genome and entered in to the lytic cycle to produce new virions and stx using host cell machinery. After assembling and forming new virus particles and stx, the host cell has been lysed and released new infective particles and stx. Again the new infective phages have been affected the phage susceptible bacteria but phage immune bacteria is safe from infective phages. The bacteriophage mediated genetic exchange mechanism is significant component of continued bacteria evolution. In this article, It has been suggested that lysogenic conversion and generalized and specialized transduction occur in nature every day on the order of >1016 times, with some events involving the transfer of virulence genes between pathogens and non-pathogens. According to, this paper, the evolution of *E.Coli 0157:H7* through complicated genetic events and that bacteriophage enhanced bacterial pathogen is a great concern in the general, public and food industry. A further example has been demonstrated that acquisition of antibiotic resistance by *P.*

aeruginosa and *Salmonella* and the researchers have been observed the higher transduction rates within the mammalian gut and in the hospital environment. So, the human life is negatively affected by the bacteriophages as the bacteriophages are host dependent exchanging genetic material to the host bacteria and converting nonpathogenic strains to pathogenic. As a result, increased virulence and other factors that negatively impact human life.

6. When using bacteriophages in antimicrobial therapy, is genetic mobilization taken into account?

Because of the multidrug resistant bacteria, phage therapy can be used as an alternative to antibiotics. But there are still some concerns and legal issues to overcome before it can be implemented on a large scale such as the bacteriophages have the ability to transfer genetic material from one bacterial strain to another in case of antibiotic resistance through horizontal gene transfer. So here focused on the problem of phage therapy in case of using complete phage particles and overcome the obstacles of using complete phage particles to establish phage therapy as an alternative to antibiotics.

Bacteriophages, or phages, play a crucial role in the regulation of bacterial population. Phages are responsible for a considerable amount of horizontal gene transfer and the evolution of their genomes is characterized by an unusually high degree of horizontal genetic exchange. The fact that phages can mobilize bacterial DNA means they can also mobilize and transduce virulence genes, antibiotic resistance genes, or genes related to fitness. Bacteriophages basically consist of one nucleic acid molecule (the phage genome) surrounded by a protein coating, the capsid. The packaging of the nucleic acid in a protein capsid confers protection and hence extracellular persistence, which cannot be found in naked DNA or RNA. Since phage-packaged DNA is protected from degradation, and phages can persist in different extracellular environments without losing their infectious capabilities. So, gene transfer through transduction by bacteriophages might be important fact. Antibiotic resistant genes (ARGs) can be horizontally transferred to other bacteria by mobile genetic elements, most commonly plasmids and transposons, although recently it has been proposed that bacteriophages are also involved. Some bacterial genera can produce phage-like elements using information encoded in their own genome called gene transfer agents (GTAs) have a bacteriophage-like capsid and that scenario has been seen in α proteobacteria that could play a role in the spread of bacterial DNA in other bacterial groups. A recent study has reported that bacteriophages (understood as complete phage particles containing phage DNA) rarely encode ARGs. ARGs occur in the bacteriophage DNA fraction of human fecal samples , hospital wastewater, aquaculture wastewater , sludge, raw

wastewater and environmental samples. Phage Lytic Proteins are the Suitable Alternative in Phage Therapy to Avoid the Risk of Genetic Transfer. Phage lytic enzymes have also been explored as antimicrobials. There are two general classes of phage lytic proteins that mediate the enzymatic cleavage of peptidoglycans (PGs): endolysins and virion-associated peptidoglycan hydrolases (VAPGHs). While VAPGHs degrade PGs in the first stages of phage infection prior to phage DNA injection, endolysins are expressed in the last stages, ensuring the release of the phage progeny via bacterial lysis. A complete phage particle can transfer the genetic content but the lytic enzymes cannot do so. Due to their high specificity and strong activity, bacteriophage lytic enzymes may be as effective as phages while offering additional benefits. Phage lytic proteins have already proven their efficacy in vitro as well as in animal models. Regarding human applications, Phase I and II clinical trials have been completed by GangaGen Inc. for the intra-nasal use of an anti-staphylococcal phage protein, and ContraFect is carrying out Phase II trials for the intravenous use of CF-301 to treat *S. aureus* bacteremia whose results have not yet been published. For topical application, Staphefekt™ developed by Micreos is the first endolysin approved for use in humans on intact skin. It is commercialized under the brand Gladskin, which includes products to treat *S. aureus* skin infections.

Phage lytic proteins are suitable to use as therapeutic because they possess many properties like their synergistic effect with other antimicrobials, their effectivity at low doses would reduce both the immune response and therapy costs, highly specific, destroying the target pathogen without affecting commensal microflora, proteinaceous in nature, noncorrosive and biodegradable etc. Several studies have reported the development of antibodies against endolysins upon systemic or mucosal application in animal models, but no adverse effects or anaphylaxis were observed and no inactivation by antibodies occurred. The therapeutic potential of phage lytic proteins has prompted the development of tailor-made antimicrobials based on these enzymes. Their unique modular structure enables domain shuffling, giving rise to antimicrobials with the desired specificity and enhanced activity. Initially, the major disadvantage of the antimicrobial activity of phage lytic enzymes was their inefficacy against Gram-negative bacteria due to the outer membrane barrier of the bacteria. But, this problem have been resolved through the development of Artilysin®. Artilysin® is an engineered enzymes that possesses both the lytic activity of a phage-derived enzyme and the outer membrane-penetrating activity of an antimicrobial Peptide.

Now-a-days multidrug resistant bacteria are available in our environment because of the overuse of antibiotics. So, to solve this problem, phage therapy can be used as alternative to antibiotic. Antibiotic resistant genes can be horizontally transferred from one bacterial strain to other bacterial strain by mobile genetic elements such as bacteriophages, plasmids, transposons. A recent study has been reported that bacteriophages (understood as complete phage particles containing phage DNA) rarely encode Antibiotic resistant genes (ARGs). ARGs containing bacteriophage DNA fraction emerge from human fecal samples, hospital wastewater, aquaculture wastewater, sludge, raw wastewater and environmental samples.

So, instead of using complete phage particle, phage lytic proteins have been used as alternative to antibiotic. Although there are some limitations and disadvantages related to phage lytic proteins but the engineering and production of the enzymes are ready to solve those limitations and disadvantages.

So, the therapeutic use of phage lytic proteins is more feasible and advisable than that of complete infective phage particles.

7. Virulence genes are transmitted by phages

The most potent toxins known to be produced by bacteria seem to be encoded by converting bacteriophages. So, the encoded converting genes of converting bacteriophages have the ability to turn the nonpathogenic strain in to pathogenic one. The pathogenicity enhanced by bacteriophages causing diseases in pants, animals and humans. Bacteriophages alter phenotype of bacteria, and in case of virulence, this is usually enhanced.

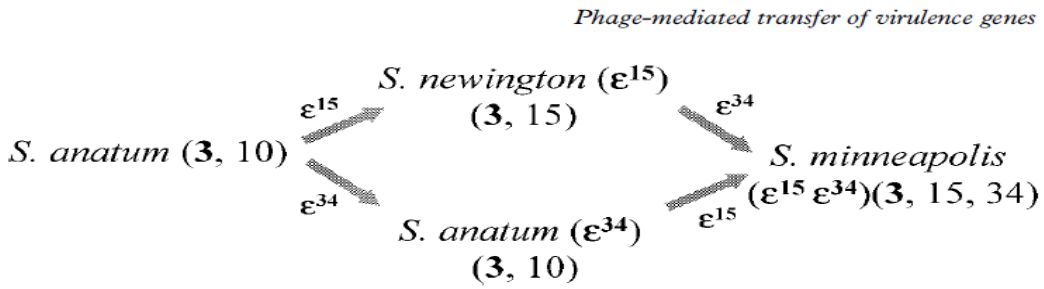

Figure 7.1: Phage mediated transfer of virulence genes.

Lysogenic conversion in the group E salmonella o- antigen is more subtle. Here, changes in the lipopolysaccharide (LPS) chemistry caused by phage encoded enzyme that modify the outer repeating trisaccharide residues result in changes in the antigenic properties that affect typing schemes. Thus *salmonella anatum* is successfully converted to *salmonella newington* and then to *salmonella minneapolis*. The order of lysogenisation is critical because D- glucosylation of galactose encoded by phage €^34. Only occurs if bacteria are already lysogenised with phage €^15 because acetylation of galactose residues in the s. anatum 3,10 antigen is blocked. Vast majority of converting bacteriophages are temperate (lysogenic). Under certain condition, that lysogenic cycle turn in to lytic cycle. A small proportion of converting bacteriophages are not temperate for example *vibrio cholerae*, carries a filamentous bacteriophage (ctx phi)
Such phages are not lysogenic but their lifestyle is such that which indicates the host is not lysed, but suffers continual shedding of mature filamentous virion and consequent reduction in growth rate. Converting bacteriophages considered as agents of "institutionalised specialised trunsduction". All virions in population of converting bacteriophages carry non-viral gene responsible for converting phenotype. Non-viral genes are not viral functional gene. Phages having tRNA act as hotspot for integration, enhance expression of virulence gene, gene expression in diverse genetic background. Carriage of virulent related genes by converting bacteriophages may arise from incidental or even accidental effects on virulence for example lambda bor genes confers serum resistance on an *E.Coli* lysogen and changes in lps may be mediated. Bona fida virulence genes are more commonly observed in converting phages.

Species	*Toxin*	*Phage properties*
Corynebacterium diphtheriae[2]	Diphtheria	35kb DS DNA
Clostridium botulinum[27]	Botulinum	110–150kb DS DNA
Streptococcus pyogenes[28]	Pyrogenic exotoxin A	~45kb DS DNA
Staphylococcus aureus[29]	Enterotoxin A	49kb DS DNA λ-like *int-xis* cluster
Vibrio cholerae[4,5]	Cholera	9.7kb SS DNA* Filamentous (M13-like)
Pseudomonas aeruginosa[30]	CTX cytotoxin	35kb DS DNA P2-like
Escherichia coli O157[20,24]	Verotoxin Enterohaemolysin	50–70kb DS DNA (λ-like) 40–43kb DS DNA (λ-like)

Table 7.2: Some examples of toxins encoded by converting bacteriophages.

Phage conversion ultimately leads to toxin production by many pathogenic bacteria and the virulent genes responsible for causing pathogenicity as well as toxin production, encoded by converting bacteriophages. Verotoxin also called shiga like toxin associated with bloody and systemic complications following enterohaemorrhagic *E.coli* infection. There are 2 parts of verotoxin such as VT1 and VT2. VT1 is identical to shiga toxin from *Shigella dysenteriae* is active primarily on gut epithelium and VT2 is less identical to shiga toxin which active primarily outside the gut e.g. in the kidney. VT are AB toxin of Cholera toxin family. VT production is almost invariably associated with lamboid VT (stx) phages and these viruses play a profound role in horizontal gene transfer and the emergence of new VTEC/STEC phenotype. Toxin production may be regulated by iron levels (fur-regulated) for some VT1 phages as in shiga toxin itself, most VT1 and all VT2 phages exhibit toxin production and therefore only express toxin when the phage undergoes lytic cycle. Inducers of VT phages enhance VT production such as mitomycin c, UV light, quinolone antibiotics induce SOS response and lead to lytic bursts and massive increase in VT production. VT production comes from DNA sequencing studies of VT phages. The genetic organization of phage DNA flanking the stx gene is conserved in VT1 & VT2 converting bacteriophages. Here the toxin genes are regulated under the control of phage lambda Q protein homologue responsible for late gene expression including holin cell lysis operon(S, R and Rz) which is necessary for the release of phage particle. VT phages transmit stx gene throughout the enterobacteriaceae. VT phages have outer membrane protein receptor.

Bacteriophages are responsible to convert the non-pathogenic bacterial strain in to pathogenic one. This is because of the bacteriophages mediated virulent genetic transfer to the bacterial strains. So, that's why bacteriophages are termed as converting phages or bacteriophages. So, in this article, here has been shown that why bacteriophages are responsible for converting nonpathogenic bacterial strains in to pathogenic, how they are modifying bacterial cell wall, how they lyse bacterial cell wall for infection etc. Most of the converting phages are temperate (lysogenic). Under certain condition, those temperate phages go to the lytic cycle and cause bacterial cell lysis and release of new copies of phages. During lytic cycle, temperate phages carrying virulence gene, amplify their virulence gene and cause infection. A small proportion of converting bacteriophages are not temperate for example vibrio cholerae, carries a filamentous bacteriophage (ctx phi). Bacteriophages do structural modification of bacteria that can help bacteriophages mediated genetic transfer to the bacterial strains. Converting bacteriophages have been considered as agents of "institutionalised specialised trunsduction". All virions in population of converting bacteriophages carry non-viral gene responsible for converting phenotype. Non-viral genes are not viral functional gene. Bacteriophages owned tRNA which act as hotspot for integration, enhance expression of virulence gene, gene expression in diverse genetic background. As shown in table 7.2 some toxin encoded and transfer by converting

bacteriophages to the bacterial strains and causing harm in humans, animals and plants. Virulent genes are responsible for causing pathogenicity as well as toxin production.

Another term, Verotoxin also called shiga like toxin associated with bloody and systemic complications following enterohaemorrhagic *E.coli* infection. There are 2 parts of verotoxin such as VT1 and VT2. VT1 is identical to shiga toxin from *Shigella dysenteriae* is active primarily on gut epithelium and VT2 is less identical to shiga toxin which active primarily outside the gut e.g. in the kidney. VTs are AB toxin of Cholera toxin family. Lamboid VT (stx) phages play important role in VT production and these viruses play a profound role in horizontal gene transfer and the emergence of new VTEC/STEC phenotype. Carriage of toxin or other virulence gene on a temperate phage allows for the possibility of gene amplification of that virulence gene during lytic cycle. Alternatively, phage carriage may provide a vehicle for rescuing essentially selfish toxin genes from doomed bacterial host cell. So, the bacteriophages use bacterial cell as host to replication, transcription, translation, assembly of virus particle, toxin production and ultimately release of new virus particles and toxin. Bacteriophages use host cell machinery, enzyme as they are host dependent.

8. Induction of phage-mediated gene transfer by fluoroquinolones in multidrug-resistant Bacteria

Fluoroquinolones are broad-spectrum antibiotics with the activity of inhibiting bacterial DNA gyrase and topoisomerase activity, which can cause DNA damage and result in bacterial cell death. In response to DNA damage, bacteria induce an SOS response to stimulate DNA repair. However, the SOS response may also induce prophage with production of infectious virions. In this study, commonly used fluoroquinolones prescribed for human and veterinary clinical disease or prophylaxis could also induce generalised transducing prophage integrated in multidrug-resistant (MDR) *Salmonella Typhimurium* DT120 and DT104. This induction of generalised transducing prophage present in *Salmonella Typhimurium* phage type DT120 and DT104 could facilitate transfer of both chromosomal and plasmid DNA, including antibiotic resistance. Here also demonstrated that fluoroquinolones can induce phage-mediated gene transfer of a native Salmonella plasmid that encodes resistance to the antibiotic kanamycin.

Three fluoroquinolones (ciprofloxacin, enrofloxacin, and danofloxacin) were investigated to determine whether exposure to any of these antibiotics would induce endogenous prophage in *S. Typhimurium* DT104 and DT120 resulting in bacterial cellular lysis. Ciprofloxacin is used for treating bacterial diseases in humans, whereas enrofloxacin and danofloxacin are used in veterinary medicine. After exposure to the three fluoroquinolones, the bacterial culture densities of BBS 1162 (DT120), BBS 1165 (DT104) and BBS 1170 (*S. Typhimurium* LT2 derivative) precipitously decreased 2 hours after antibiotic addition (data not shown); the decrease in culture density was similar to *S. Typhimurium* exposed to carbadox. The three strains contain a plasmid that is native to MultiDrug-Resistant *S. Typhimurium* DT104-745 and confers kanamycin resistance that means the three strains contain a plasmid that is native to Multi Drug Resistant *S. Typhimurium* DT104-745, is a kanamycin antibiotic resistant plasmid. Now, fluoroquinolone-induced phage lysates were performed phage transduction from the three strains into a kanamycin-sensitive recipient (BBS 243). BBS 243 is a kanamycin sensitive strain. For BBS 1162 and BBS 1165, all three antibiotics (ciprofloxacin, enrofloxacin, and danofloxacin) stimulated generalized transduction of the kanamycin resistance plasmid into BBS 243 (Table 8.1).

Donor	Kanamycin transductants of BBS 243/mL of lysate (mean ± S.E.M.)			
	Ciprofloxacin 0.1 μg/mL	Enrofloxacin 0.5 μg/mL	Danofloxacin 0.5 μg/mL	No fluoroquinolone
BBS 1162	494 ± 111	275 ± 126	500 ± 194	0 ± 0
BBS 1165	1013 ± 290	750 ± 109	808 ± 74	4 ± 2
BBS 1170	0 ± 0	0 ± 0	0 ± 0	0 ± 0

S.E.M., standard error of the mean.

Table 8.1: Average generalized transduction frequency following fluoroquinolone induction

So, it is clear to understand that these fluoroquinolones enhance horizontal gene transfer from MDR *S. Typhimurium* to a susceptible bacterial host strain. On the contrary, it has been seen that fluoroquinolone exposure of BBS1170 did not cause antibiotic-induced generalized transduction because although *S. Typhimurium* LT2 harbors several prophages in its genome but it does not contain a prophage that confers generalised transductions such as bacteriophage P22. BBS 1170 strain is a *S. Typhimurium* LT2 derivative. Exposure of fluoroquinolones induces increase the expression of recA gene and that gene is responsible for activation of bacterial SOS response. Ultimately the SOS response induces prophage with production of infectious virions. Fluoroquinolone exposure increases the transcription of specific genes for prophage activation. Real-time PCR was performed to measure the relative transcript levels of recA and two Prophage-1 (P22-like) genes, abc2 and kil, in BBS 1162 prior to the addition of ciprofloxacin as well as 60 min and 120 min following antibiotic exposure. At 60 min and 120 min, expression of the three genes were significantly up regulated in response to ciprofloxacin (Fig. 8.2; $P < 0.01$). In the no-antibiotic control cultures, the log2 expression of recA, abc2, and kil either did not change or slightly decreased from the initial time point to the 60 min and 120 min time points. In the response of ciprofloxacin exposure to LT2, transcription of the recA, abc2, and kil genes were also measured. recA expression was significantly up regulated at 60 min and 120 min, but no abc2 and kil gene expression was detected because LT2 does not contain Prophage-1(P22). So, the addition of ciprofloxacin increases expression of recA gene which activates the SOS response in bacterial strain. Furthermore, increased expression of genes abc2 and kil of prophage-1 in BBS 1162 strain is indicating that fluoroquinolone exposure induced generalized transducing prophage to facilitate antibiotic-induced horizontal gene transfer.

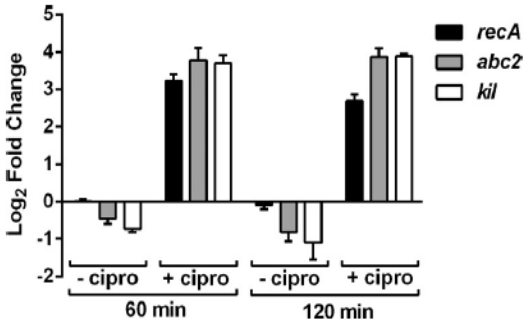

Figure 8.2: Ciprofloxacin exposure increases gene expression of recA, abc2, and kil. Real-time gene expression of recA, abc2, and kil in BBS 1162 following 60 min and120 min with and without ciprofloxacin (0.1 µg/mL) addition at the 0-min time point (OD600= 0.2). The log2fold change is in comparison with the 0-min time point, with significant differences in expression of all three genes at both time points following ciprofloxacin exposure ($P < 0.01$). OD600, optical density at 600 nm.

Fluoroquinolones are broad-spectrum antibiotics. Owing to their wide spectrum of activity against a variety of bacterial pathogen, fluoroquinolones are important for human clinical and veterinary therapy. The use of antibiotics in human and veterinary medicine may have unintended consequences. So, the exposure of antibiotic can cause antibiotic resistance, antibiotic associated diarrhea, enhanced pathogen invasion and stimulation of horizontal gene transfer. In this experiment, certain Multi Drug Resistant strains of *S. Typhimurium* DT120 and DT104 exposed to fluoroquinolones have been shown their decreased culture density and induced phage-mediated gene transfer including the transduction of a plasmid native to Salmonella that confers kanamycin resistance. Fluoroquinolones are an important class of antimicrobials that are used to treat a variety of infectious diseases both in humans and animals. Exposure of fluoroquinolones induces endogenous prophage in *S. Typhimurium* DT104 and DT120 through increasing the expression of bacterial recA gene which induces the activity of bacterial SOS response. That SOS response not only stimulate bacterial DNA repair that has been damaged by fluoroquinolones but also induce prophage with production of infectious virions. And also, fluoroquinolone exposure suggests that the transcription of specific genes for prophage activation may also be increased. The research has been reported here, after exposure of ciprofloxacin, there have been increased the expression of recA, abc2 and kil genes of BBS 1162 strain containing P22 prophage. In another research, there has been found that bacterial exposure to fluoroquinolones induces prophage-associated Shiga toxin (Stx) in *E. coli* O157:H7 and the horizontal gene transfer in *Staphylococcus aureus* and *Vibrio cholera.* For the veterinary fluoroquinolones such as enrofloxacin and danofloxacin to be administered in animal production situations where Salmonella strains are unknowingly present. For example, danofloxacin is suggested for the treatment of bovine respiratory disease due to both Mannheimia haemolytica

and Pasteurella multocida. Enrofloxacinis suggested for respiratory and enteric diseases. In addition, the animal production environment could be contaminated with a strain of salmonella which can perform generalized transduction. Excretion of fluoroquinolones occur in treated animal's urine and feces which can contaminate the production settings where salmonella is present that can induce prophage to enhance horizontal gene transfer. Antibiotic withdrawal before completing proper dose can create problem. Diard et al. have been demonstrated an experimental evidence. Mice colonized with *S. Typhimurium*. There has been suggested that treatment with two doses of ciprofloxacin 8 hours apart eradicated the pathogen from the gut lumen. But withdrawal of antibiotic can create tissue-associated *S. Typhimurium* recolonization of the gastrointestinal tract. So, this research provides a wide list of scientific investigations which indicate that fluoroquinolones exposure to humans and animals can stimulate horizontal gene transfer in bacterial populations. Physicians and veterinarians should consider adverse effect, unintended consequences when determining antibiotic therapy for the treatment of infectious diseases. They should also discuss with their patients about the bad impact of over dose use, incomplete dose use, improper timing of antibiotic usage and not throwing antibiotic related or wastage everywhere.

9. Conclusion

So, the bacteriophages horizontally transfer various types of harmful genetic materials that create threat to human life. Antibiotic resistant bacteria are available and increasing day by day. The mobile genetic elements like phages, plasmids, transposons are playing important role for spreading antibiotic resistant bacteria. The foodborne bacteria are responsible for causing foodborne diseases and their pathogenic activity of the foodborne bacteria enhanced by the temperate bacteriophages. Infectious bacteriophages normally lysogenize new bacteria and transform non-STEC bacteria to STEC. Multidrug resistant bacteria are another problem for human life because of the overuse of antibiotics. So, here phage therapy can be used as an alternative to antibiotic. As an alternative to antibiotics, phage lytic proteins have been utilized instead of whole phage particles. Although there are some limitations and disadvantages associated with phage lytic proteins, the engineering and production of the enzymes are ready to overcome those limitations and disadvantages. As a result, the therapeutic use of phage lytic proteins is more feasible and recommended than the use of complete infective phage particles. Bacteriophages are responsible for transforming non-pathogenic bacteria into pathogenic ones. This is due to bacteriophages' ability to convey pathogenic genetic information to bacterial strains. As a result, bacteriophages are often referred to as converting phages or bacteriophages. Temperate phages containing virulence genes enhance their virulence gene and cause infection during the lytic cycle. A limited percentage of converting bacteriophages, such as Vibrio cholerae, are not temperate. Bacteriophages alter the structure of bacteria in order to facilitate bacteriophage-mediated genetic transmission to bacterial strains. Use of antibiotic fluoroquinolones could also induce generalised transducing prophage integrated in multidrug-resistant bacteria. Fluoroquinolones are broad-spectrum antibiotics. Owing to their wide spectrum of activity against a variety of bacterial pathogen, fluoroquinolones are important for human clinical and veterinary therapy. The use of antibiotics in human and veterinary medicine may have unintended consequences. So, the exposure of antibiotic can cause antibiotic resistance, antibiotic associated diarrhea, enhanced pathogen invasion.

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