Horizontal Gene Transfer: An Insight into Antimicrobial Resistance

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Declaration

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Abstract

Antimicrobial resistance (AMR), which affects a variety of infectious pathogens, is a pivotal public health issue for many countries and corporations. Governments across the globe are starting to take heed of an issue that is so grave that it jeopardizes the advancements of advanced medication such as antibiotics. A key factor in the spread of antibiotic resistance is horizontal gene transfer (HGT). Contrarily, it is periodically presumed that antibiotics stimulate HGT. A comprehensive review of the research, however, indicates that there is insufficient credible data available to support such a hypothesis in principle. This is primarily due to the insufficiency of concise quantitative studies to answer this query. We assess how significantly HGT correlates to the antibiotic resistance spread in this study, as well as what is documented about how antibiotics regulate the mechanics of HGT. Our attention is on conjugation, the predominant HGT method that is primarily responsible for the global spread of antibiotic resistance. According to our research results, trials to quantify HGT must be planned in a systematic way in order to critically evaluate the outcomes. Such experiments are crucial for constructing cutting-edge approaches to inhibit the propagation of resistance by HGT. In this study, we explore about how much HGT contributes to the spread of antibiotic resistance and explore what is known about how antibiotics affect the dynamics of HGT including a brief discussion on antibiotic resistome.

Keywords: antibiotics, antibiotic resistance, horizontal gene transfer

Introduction

Antimicrobial substances that are effective against bacteria are antibiotics. Since antibiotics are quite frequently utilized in both the treatment and prevention of bacterial infections, they are the most crucial form of antibacterial agent for countering such infections since bacterial growth may be inhibited or even eliminated by them. Antibiotics uphold modern medicine; they are essential for invasive surgery and therapies like chemotherapy and have decreased pediatric mortality and improved life expectancy.

However, all kinds of antibiotics now used in clinical settings are now facing resistance. Antibiotic resistance has been shown to be inevitable, and it frequently develops promptly after an antibiotic is introduced to a clinic. Determining the causes, extent, and development of antibiotic resistance is consequently of significant importance (Wright, 2010). Anti-bacterial, anti-parasitic, anti-viral, and anti-fungal medication efficacy is reduced considerably due to AMR, making patient treatment challenging, expensive, or perhaps unattainable. The effects on highly susceptible individuals are most evident, leading to chronic illness and a greater mortality rate (Mazaheri Nezhad Fard et al., 2011).

Antibiotic resistance in bacteria, particularly multidrug resistance (MDR), has emerged as a major problem that threatens the safety of the environment, food, and both human and animal health. By 2050, it is predicted that MDR will be responsible for 10 million human fatalities, outnumbering those caused by cancer (World Health Organization, 2014). MDR bacteria are becoming more common in diseases across the world, and the threat of untreatable infections is beginning to develop. A major hazard to human health, according to the most recent World Economic Forum Global Risks assessments, is antibiotic resistance (Blair et al., 2014). In addition, to be degraded resistance to antibiotics through chromosomal gene changes and horizontal gene transfer, bacteria can also be innately resistant to certain antibiotics. A bacterial species' capacity to withstand the effects of an antibiotic due to innate structural or functional traits is known as intrinsic resistance.

In contrast to the vertical inheritance of genes from parents to offspring, lateral or horizontal gene transfer (HGT) describes the exchange of genetic material across or within species. Bacteria

can acquire new genetic material either naturally through internal genetic mutation or indirectly through HGT. After being discovered in 1928 (Griffith, 1928), HGT is becoming more well recognized as a major driver in microbial evolution as it allows bacteria to acquire complex new traits (Boto, 2009; Ragan & Beiko, 2009). According to estimates, previous HGT phenomena caused the derivation of 17% of the Escherichia coli genome and up to 25% of genome of other bacterial species (Ochman et al., 2000).

There are three primary mechanisms by which HGT occurs in bacteria: conjugation, transformation, and transduction (Stewart, 2013). For conjugation to occur, the donor and recipient cells must come into direct contact. The donor cell creates a multiprotein bridge that connects the mating pair of cells, allowing DNA to be transferred between them. Most of the time, the DNA that has to be transferred is single-stranded in the donor cell before being converted to double-stranded DNA through replication process of the recipient cell. The bulk of newly found conjugative systems are plasmid-encoded, and DNA from plasmids serves as their conventional substrate (Wozniak & Waldor, 2010). But it has recently become clear that conjugation mechanisms are common in chromosome-borne mobile genetic components (MGEs) as well. The terms integrative and conjugative elements (ICEs) are frequently used to describe such components (Wozniak & Waldor, 2010).

In transformation, extracellular naked DNA is absorbed by cells that have acquired genetic competence, horizontal gene transfer (HGT) is one method used by bacteria. Transformation may be distinguished from DNase-resistant HGT processes by their sensitivity to DNase, which breaks down bare DNA (Hasegawa et al., 2018). Meanwhile, bacteriophages facilitate gene transfer during transduction (Ozeki & Ikeda, 1968). A broad variety of sequences, including antibiotic resistance cassettes, may be transferred by transduction and transformation (Lopatkin et al., 2016). Following precise identification between the phage and its corresponding receptor on the bacterial surface, transduction takes place. Since many phages have a limited host range due to their specialization, conjugation is thought to have had a greater impact on the spread of antibiotic resistance (Smillie et al., 2010). Natural transformation frequently takes place in a brief physiologically competent condition brought on by environmental signals like nutrient availability or cell density (Thomas & Nielsen, 2005). Even though bioinformatic investigations show that most bacteria have genes that are similar to known competence genes (Johnsborg et

al., 2007), it is uncertain if these bacteria go through transformation (Mell & Redfield, 2014; Johnston et al., 2014). As a result, studies that claim there are not many naturally transformable species tend to believe that transformation does not significantly contribute to the dissemination of antibiotic resistance genes, albeit more research is necessary (Lopatkin et al., 2016).

Genes that have been horizontally transmitted can encode many different properties, including as metabolic characteristics, virulence components, and antibiotic resistance (Lopatkin et al., 2016). Resistance-carrying MGEs are part of the shared gene pool used by a variety of microorganisms (Norman et al., 2009). The transmission of resistance genes can be significantly sped up by HGT since it can traverse phylogenetic barriers (Hawkey & Jones, 2009). In fact, it has been hypothesized that HGT has made it possible for resistance to most commercially available antibiotics to spread (Bennett, 2008; Davies & Davies, 2010; de la Cruz & Davies, 2000). On the other hand, the usage of antibiotics may have an impact on HGT dynamics and, as a result, the emergence of antibiotic resistance. An antibiotic, however, has the potential to both selectively modify the general dynamics of HGT and the efficiency of HGT at the single-cell level. Experiments must be carefully designed in order to separate these various impacts in order to fully understand these effects (Lopatkin et al., 2016).

The resistome is a complex collection of genes whose actions either directly or indirectly work to inhibit antibiotic activity. Combinatorial resistance is oftentimes caused by the presence of many processes in resistant strains, which further complicates the issue and makes it complex to understand how each process affects the entire phenotype in regards to clinical treatment (Institute of Medicine et al., 2011; Wright, 2010).

Antibiotic Resistance transmission via Conjugation

Conjugation is the process by which DNA is transferred from one cell to another via cell surface pili or adhesins. The conjugative mechanism, which is either encoded by genes on plasmids capable of independent replication or through integrative conjugative components in the chromosome, facilitates it (Wozniak and Waldor, 2010; Smillie et al., 2010). Furthermore, as seen for the very broad host range IncQ plasmids, this conjugative mechanism might permit the mobilization of non-conjugative plasmids (Meyer, 2009). Conjugation is unquestionably the best researched of the several processes that may enhance HGT (Figure 1) (Guglielmini et al., 2013; Norman et al., 2009). In many instances, conjugative elements like plasmids or transposons are linked to ARGs. Although these components can also be transferred by transformation and perhaps even transduction, conjugation is frequently regarded as the most plausible causal agent. This is because it offers superior environmental defense, a more effective method of accessing the host cell over transformation, and frequently a wider host spectrum than bacteriophage transduction. Additionally, whereas the process of conjugation is intended to transmit bacterial genes, the transfer of bacterial DNA through transduction is a byproduct of incorrect bacteriophage replication (Norman et al., 2009).

Given the extensive research in this field on human infections, it is evident that after resistance genes successfully establish themselves onto viable plasmids, they may swiftly spread through various strains as well as species (von Wintersdorff et al., 2016). The bla_{CTX-M} ESBL genes, that have spread across several Enterobacteriaceae plasmids with both restricted and wide host ranges in addition to various opportunistic human infections, serve as an excellent example of this (von Wintersdorff et al., 2016; Canton et al., 2012). Since these genes have become incredibly common in people, animals, and the ecosystem (Woerther et al., 2013; von Wintersdorff et al., 2016; Hartmann et al., 2012). Additionally, the transfer of plasmids in infections has caused the dissemination of multiple ARGs that code for resistance to aminoglycosides, β-lactams, sulfonamides, tetracyclines, quinolones, and many other drug classes around the world (von Wintersdorff et al., 2016; Huddleston, 2014). A growing number of reports of the spread of plasmids containing carbapenem resistance (Carattoli, 2013) and the recent identification of plasmid-encoded colistin resistance in China (Liu et al., 2015) are of particular concern. These developments may lead to Enterobacteriaceae becoming truly pan-drug resistant (Arcilla et al.,

2015). Additionally, numerous ARGs are frequently co-localized on the same plasmid, thus makes it possible for multidrug resistance to propagate quite quickly (von Wintersdorff et al., 2016).

Among the three types, the far more complex type of horizontal gene transfer (HGT) that occurs in bacteria is conjugation, and it offers a platform for the dissemination and maintenance of virulence and antibiotic resistance genes (von Wintersdorff et al., 2016; Norman et al., 2009). Integrative conjugative elements (ICEs) and conjugative plasmids (CPs) serve as the transfer carriers (ICEs). Different forms of CPs can be distinguished using phylogenetic analysis. a group of conjugative plasmids classified as F-like because, in terms of their DNA transfer genes, they are related to the well-established F plasmid or F, or F-factor. Since Lederberg and Tatum's (LEDERBERG & TATUM, 1946) first depiction of bacterial conjugation, F and F-like plasmids plays a major role in elucidating the molecular processes and architectures enabling DNA transfer between bacterial cells. While F-like plasmids appear to be confined to closely related enterobacterial genera like Citrobacter, Enterobacter, Escherichia, Klebsiella, Salmonella, and Shigella conjugation is pervasive throughout the bacterial and archaebacterial world (as are ICEs and CPs) (Koraimann, 2018). These facultatively anaerobic, Gram-negative bacteria may thrive in a variety of environments, including the gastrointestinal tracts of people and animals, where they can live commensally or cause moderate to severe illnesses.
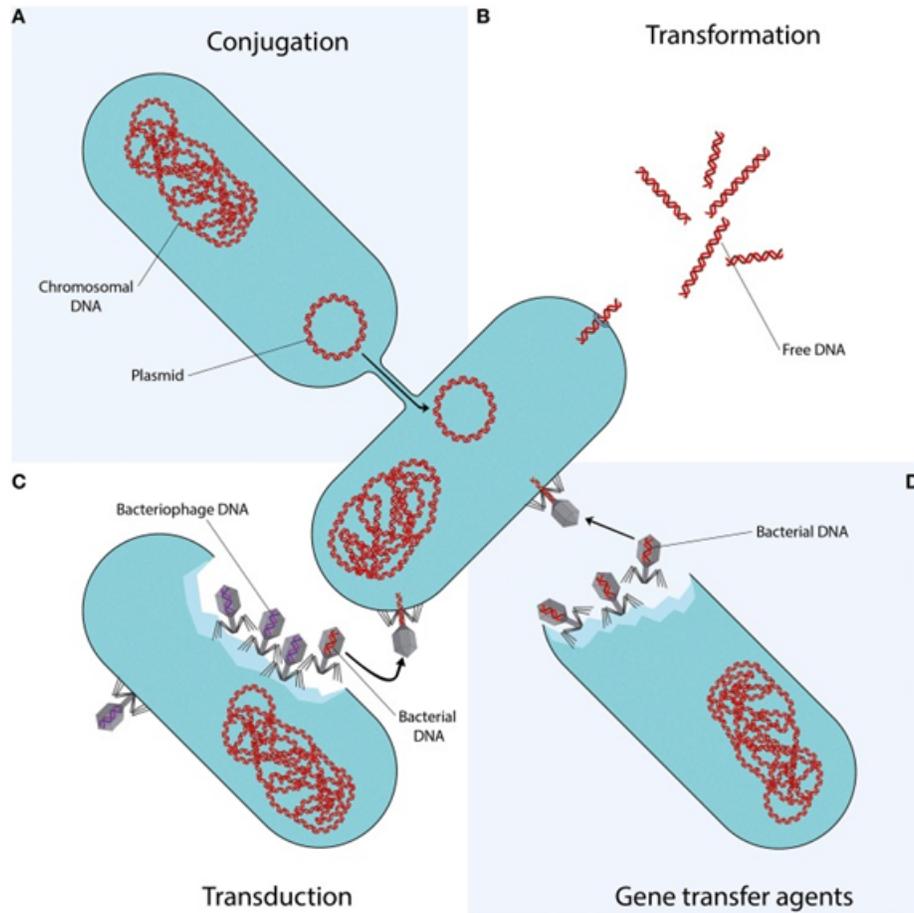
Figure 1: Horizontal gene transfer mechanisms. The technique of gene transfer represented by each quadrant is distinct. (I) The process of conjugation, in which DNA is transmitted from the donor cell to the recipient cell, requires cell to cell contact via cell surface pili or adhesins. In (II), transformation, bare extracellular DNA fragments are taken up, incorporated, and functionally expressed. (III) Bacteriophages can transmit bacterial DNA from an infected donor cell to an uninfected target cell by specialized or generic transduction. Bacterial DNA may inadvertently be injected into the phage head during generalized transduction (represented as a phage with a red DNA strand.). It is co-extracted and loaded into a new phage during specialized transduction of genomic DNA that is close to the prophage DNA (not represented). (IV) Bacteriophage-like particles called gene transfer agents (GTAs) transport random genome fragments from the generating cell. The transfer of GTA particles to a recipient cell might occur as a result of cell lysis (von Wintersdorff et al., 2016).

The worldwide resistance landscape is shaped in part by HGT and in part by antibiotic selection (Martínez, 2008; Aminov, 2009). In a limited setting, such as a patient's intestines, HGT facilitates the local dissemination of resistance genes across an origin species to different strains or species (Karami et al., 2007; Egervärn et al., 2009). People, animals, and objects that contain newly resistant germs are continually shifting and interacting. Due to this enhanced diffusion, the newly acquired resistant bacteria can spread globally. Through HGT, resistant bacteria spread the resistance determinants farther in new situations. By enhancing for the resistant offspring, the use of antibiotics might enhance the overall impact of HGT. Selection may increase the number of cells competent of HGT, increasing the likelihood of more HGT events (Stecher et al., 2012). Collectively, these factors have undoubtedly assisted resistance expand across the globe (Stokes & Gillings, 2011; Berendonk et al., 2015).

The proliferation of β-lactamase (Bla) varieties serves as the finest illustration of this. Among the earliest and most frequently given antibiotic classes, β-lactams, are resistant to bla enzymes (Davies & Davies, 2010). Numerous bla genes presumably propagate by conjugation (Jacoby & Sutton, 1991; Johnson & Woodford, 2013), and bla variations, particularly extended-spectrum β-lactamases (ESBLs) (Meini et al., 2014), are frequently encoded on plasmids (Shaikh et al., 2015; Vaidya, 2011). For instance, lateral acquisition is suggested by MGE-associated CTX-M (a frequent bla variation) in Enterobacteriaecae species (Olson et al., 2005), which shares adjacent gene sequences with Kluyvera species. Similar to this, the variety of species bearing conjugative plasmids with the sequence-identical metallo-β-lactamase bla_{NDM-1} (Table 1) which confers resistance to carbapenem medicines, implicates the significance of conjugation. In fact, one investigation from New Delhi's sewage and water faucet discovered the prevalence of bla_{NDM-1} in upwards of 20 different strains, many of which previously had not been connected to ESBL resistance; each isolate is capable of transmitting their plasmid through conjugation under specific experimental circumstances (Walsh et al., 2011). The occurrence of such cases has probably been aided by the strong β-lactam selection pressure during the past few decades.

Numerous resistance genes are found in human gut microbiota (Bailey et al., 2010; Marshall et al., 2009), and this microbiota can serve as an accumulation for other dependent or pathogenic bacteria to pick up resistance through horizontal gene transfer (Liu et al., 2012; Huddleston, 2014; SALYERS et al., 2004). By enhancing for low-level resistance already existent, antibiotics

can create an environment that is conducive to transmission (Karami et al., 2007; Stecher et al., 2012). If two (or more) of the selected genes are physically connected to the same MGE, this selection may also affect how frequently organisms develop resistance to other drugs. In the gut flora of children who received non-FQ antibiotic treatment, for instance, one research found elevated frequencies of qnr-mediated FQ resistance (Vien et al., 2012). According to the environment, however, different elements could be what drives resistant landscapes. According to one investigation, the main factor influencing the resistance landscape in unmanicured soil conditions is not HGT but rather the makeup of the bacterial population (Forsberg et al., 2014).

Considering how frequently resistance is passed along via conjugation, it becomes plausible to anticipate that using antibiotics helps increase HGT rates. It is undeniable that the use of antibiotics in both human and animal medicine has altered the ecology of resistance (Martínez, 2008), and that this shift in the ecology of resistance has presumably had an impact on HGT by altering the number of species that carry resistance on MGEs (Baquero et al., 2013). Antibiotics may potentially modify the rate of HGT by favoring cells with higher transfer efficiencies (Stokes & Gillings, 2011). Antibiotic usage does not necessarily lead to the emergence of antibiotic resistance, though. For instance, ESBL genes have developed and propagated through conjugation in the course of millions of years prior to the current use of antibiotics (Barlow & Hall, 2002; von Wintersdorff et al., 2016). A complex issue that depends on a number of variables is the degree to which antibiotics modify the HGT rate. The concepts of HGT rate must be made clear, and trials must be well planned to yield definitive findings, in order to shed light on this subject (Lopatkin et al., 2016).

Kinetics of the conjugation reaction

Conjugation may be modeled at the population level as a biomolecular reaction between the donor (D) and the receiver (R), culminating in the transconjugant (T), where D, R, and T each signify the corresponding cell densities (Lopatkin et al., 2016; Levin et al., 1979). T's rate of generation through the two parents can be expressed as:

$$\frac{dT}{dt} = \eta \qquad\qquad (1)$$

Here the conjugation efficiency, denoted by the symbol η, is the interaction's rate constant.

If R and D are generally constant and T is largely produced by the two parents (i.e., secondary conjugation by T is minor) within a brief window of conjugation (Δt), the conjugation efficiency may be estimated as follows (Lopatkin et al., 2016):

$$\eta \approx \frac{T}{DR\Delta t}$$

The conjugation readout has previously been described as the ratio of T between two experimental conditions (for instance, with and without antibiotics) or as the relative frequency, defined as T per either parent *(T/D or T/R)*, relative increase, defined as T per either initial parent density *(T/D_i or T/R_i)* (Sørensen et al., 2003). Regardless of the nomenclature, it is possible to ascertain if certain elements contribute to the reported rise in T by comparing the conjugation readouts under various settings (Lopatkin et al., 2016).

However, as is conclusive from (1), these alternative measurements may confuse various influences on T. T in particular is produced either by cell division or conjugation. Therefore, various experimental settings (i.e., shifting D or R starting values, Δt) or antibiotic-mediated selection (growth/death of D, R, or T) can likewise cause a change in T. When evaluating if a factor such as an antibiotic does, in fact, alter the conjugation efficiency η, these effects should be avoided or reduced (Lopatkin et al., 2016).

Quantification of antibiotics' effects on conjugation

Two factors—the pace of conjugation or the conjugation efficiency and the subsequent expansion of transconjugants—affect the overall dynamics of conjugation (Figure 2 a) (Lopatkin et al., 2016). An antibiotic may influence these dynamics by altering the efficiency of conjugation, acting as a selection factor that influences the dynamics of the population after conjugation, or by doing both (Figure 2 b, c). Limitations of decoupling among these two aspects in earlier research made it difficult to quantify the impact of antibiotics on conjugation efficiency (Rensing et al., 2002; Johnsen & Kroer, 2007; Sørensen et al., 2005). Consequently, it is rather unclear whether or whether antibiotics facilitate conjugation (Blázquez et al., 2012). Generally, transconjugants have been quantified after a growth phase has been allowed to pass during in vitro conjugation studies in the presence of an antibiotic (Schuurmans et al., 2014; Smet et al., 2010). Based on the observed rise in transconjugants in the presence of an antibiotic compared to the untreated control (Barr et al., 1986), it has been concluded that antibiotics stimulate conjugation. Unfortunately, this experimental setup cannot discriminate between the antibiotic's effects on conjugation efficiency as well as its impacts on selection dynamics, and it also does not demonstrate how antibiotic concentration could affect these effects. Results from in vivo and case studies have also suggested a connection between antibiotic use and conjugation-mediated transfer of resistance, presumably because antibiotic choice creates a conducive environment for transfer (Goren et al., 2010; Karami et al., 2007; Cavaco et al., 2008). Due to the complexity of the research, much like with in vitro studies, this theory has yet to be proven beyond a reasonable doubt.
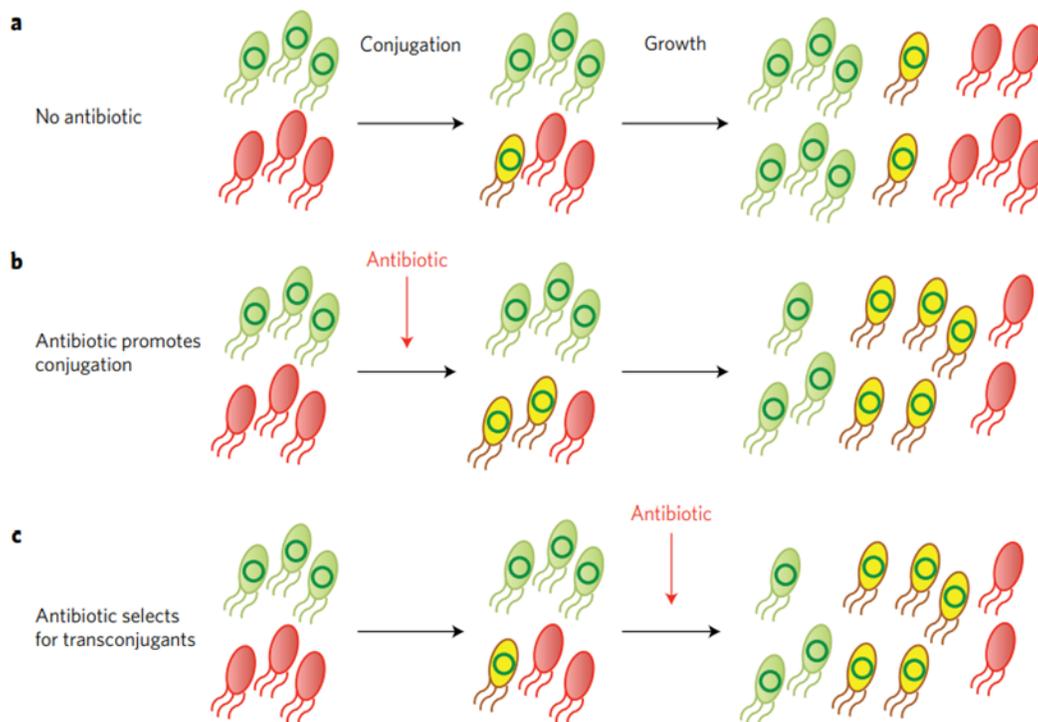
Figure 2: Antibiotics' potential impact on conjugation. a, Overall, there are two stages to conjugation dynamics: conjugation and the subsequent changes in the populations of the donor (green), recipient (red), and transconjugant (yellow). b,c, Antibiotics can change the population dynamics by modifying the growth rates of single or multiple populations (c), or they can modify the conjugation efficiency (b). In this example, we presume that, generally, antibiotics improve conjugation (b,c), relative to the untreated state (a) (Lopatkin, Huang, et al., 2016).

It's important to tell the difference between an alteration in and one in R, D, and T when evaluating antibiotic effects on conjugation. The latter may be brought on by a confluence of the possible impact of antibiotics on and the ensuing processes of selection (Rensing et al., 2002; Johnsen & Kroer, 2007; Lopatkin et al., 2016; Smets & Barkay, 2005). Antibiotics may indirectly or directly encourage (Figure 3). Antibiotics are thought to cause a general cellular response that indirectly promotes or inhibits conjugation when transfer mechanism expression is unrelated to antibiotic treatment. Antibiotics have the ability to directly induce a series of molecular activities that lead to the development of transfer machinery (Andersson & Hughes, 2014; HASTINGS et al., 2004).
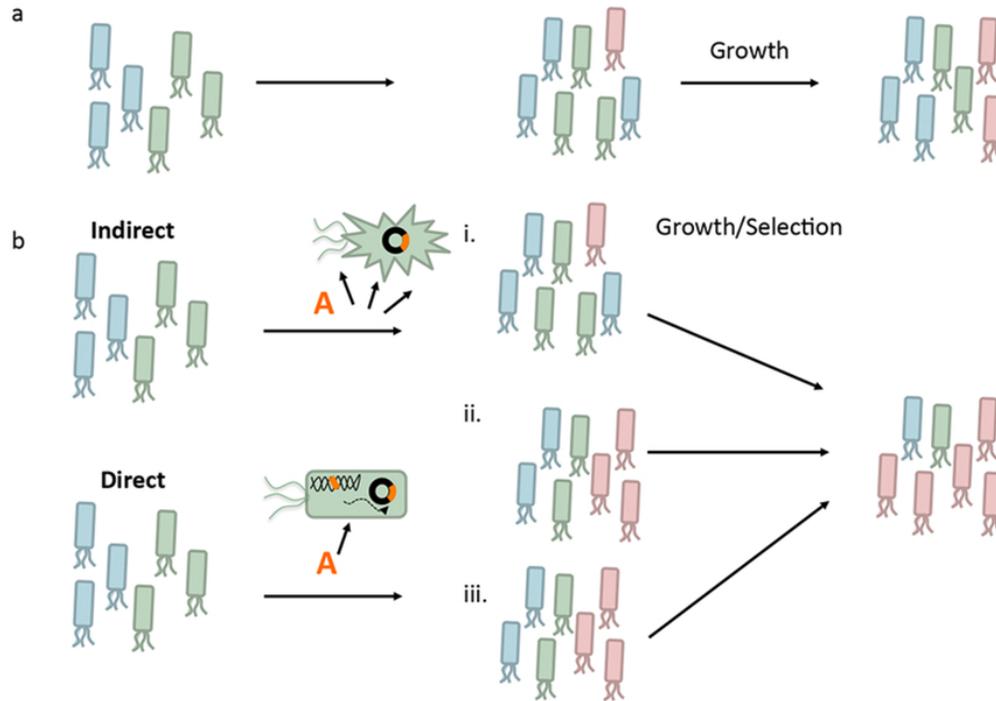
Figure 3: Prospective effects of antibiotics on conjugation dynamics. a. Conjugation happens at a certain pace in the lack of antibiotics, and the resistant progeny develop normally but are not chosen for. b. There are two hypothesized possibilities for how antibiotics alter conjugation efficiency when present. Antibiotics are believed to indirectly modify the conjugation efficiency in the first case. In this instance, antibiotics cause the cell to react universally. In these situations, it is feasible that the efficiency has either (i) increased or (ii) decreased in comparison to (a), but following a time of development and selection, all three possibilities lead to the same conclusion. Antibiotics directly induce the development of the conjugation mechanism in this scenario (iii), which is demonstrated by the excision of the resistance gene from the chromosome into a circularized plasmid. This is a typical method for integrating conjugated elements and transposables with a partner (ICE). When this occurs, the conjugation efficiency rises even before selection takes place (Lopatkin, Sysoeva, et al., 2016).

Previous research on these topics has produced what appear to be inconsistent findings (Blázquez et al., 2012), such as the fact that the same or comparable antibiotics might have different effects depending on the experimental setting. Transconjugants are often tested after a period of time Δt to assess the effects of the antibiotic. Typically, donors and recipients are combined in the presence of an antibiotic (or other stimuli) (Lopatkin, et al., 2016). The combination of sub-inhibitory kanamycin (Kan) and streptomycin (Strep) doses increased conjugation for three separate conjugative plasmids in E. coli, according to one study's T/R_i calculations (Lopatkin, et al., 2016; Zhang et al., 2013).

However, a rigorous analysis of the data suggests that the measure T/R_i, which revealed on the time-dependent expansion of the transconjugant population, may have been more accurate in reporting the conjugation efficiency improvement. Antibiotics in particular may have had a considerable influence on growth rates. As time passes and cells multiply, these variations become more pronounced. In fact, there were hardly any variations between the readout with and without treatment during the first four hours after conjugation, once these influences of antibiotic-mediated selection were probably least noticeable. Similar findings have been reported in other investigations (Zhang et al., 2013; Al-Masaudi et al., 1991; Barr et al., 1986), which link an increase in frequency to the antibiotic's indirect induction of conjugation. It is less obvious if the conjugation efficiency also increased.

It is generally known that conjugation contributes significantly to the spread of antibiotic resistance on a global scale. Contrarily, it's not always correct to say that using antibiotics encourages conjugation. In general, mechanical, physiological, and selective hurdles must be surmounted for conjugation to succeed (Smets & Barkay, 2005; Thomas & Nielsen, 2005). In fact, the findings by Lopatkin et al. suggest that there may be two reasons why the role of antibiotics to the development of conjugation is overstated (2016). Initially, when the conjugation mechanism is expressed constitutively in a system, antibiotics do not appreciably improve conjugation efficiency. Second, only a tiny group of characteristics encourage a rise in the percentage of transconjugants, even when selection dynamics are taken into account. This unexpected result results from the conflicting functions an antibiotic performs during conjugation. If an antibiotic has no impact on either of the parent strains, there is no selective advantage for the transconjugant. If the antibiotic inhibits one or both of the parent strains, the

transconjugant does get a selection advantage. To counteract the effects of positive selection on the transconjugant, the antibiotic may still reduce the frequency of conjugation by decreasing the ratios of one or both parental populations. The results of the study by Lopatkin et al. suggest that other factors, particularly the physiological state of cells prior to conjugation as well as energy accessibility throughout conjugation, have a significant impact on conjugation efficiency even when the presence of an antibiotic need not significantly increase conjugation efficacy intrinsically (Figure 2 c) (2016). Population structure predominates when selection processes are taken into account to forecast the appearance of transconjugants. The findings highlight the significance of assessing microbial population growth patterns (either with or without antibiotic treatment), in order to assess cell physiology and determine the impacts of antibiotic-mediated selection (Lopatkin et al., 2016). The evaluation of the risk posed by the spread of resistance can also benefit from this knowledge. For instance, a recent study (Korem et al., 2015) showed that it is possible to use sequencing to estimate the abundances and growth rates of various microbial communities. Quantitative assessments of the level of HGT in various natural habitats may be made using these data along with *in vitro* estimations of conjugation efficiency.

HGT quantification in dynamic conditions

Quantifying HGT and the impact of antibiotics on HGT becomes more difficult when settings are more complicated, like those of natural soils, toxic water, or biofilms (Bellanger et al., 2014; Aminov, 2011). However, it is difficult to accurately evaluate the impact of a medication on growth rates, as well as the process of gene transfer is affected by geographic dispersion and motility. These challenges make it difficult to quantify conjugation in these conditions. To differentiate between various antibiotic effects, appropriate control studies must be created. Studies demonstrating that antibiotics may increase conjugation efficiency in purified and activated sludge cultures (Kim et al., 2014) and decrease it in sewage water (Ohlsen et al., 2003) may be deceptive without these data. These findings collectively imply that HGT rates might vary based on the environment. When exposed to sub-inhibitory quantities of Kan (instead of imipenem, a -lactam), another study found increased conjugation in biofilms of plasmids expressing Kan resistance (Ma & Bryers, 2012). The authors, however, fail to distinguish between an impact on conjugation efficiency and antibiotic-induced modifications to the dynamics of biofilm growth. Growth rate predictions were ignored while quantifying

conjugation, which was additionally complicated by the interchangeability of several conjugation measures. Therefore, it would be premature to draw the inference that bacteria may "detect the antibiotics to which they are resistant" and, in turn, enhance the horizontal transmission of resistance genes (Lopatkin et al., 2016). The outcomes of these investigations may more indicate how antimicrobials affect biofilm development or community makeup than how they affect gene swapping. In order to understand how antibiotics, alter conjugation dynamics in such settings while accounting for compounding variables, new experimental methodologies and quantitative metrics need be put into place; several research have in fact started to go in that direction (Jutkina et al., 2016).

The measurement platform for conjugation in experiments

By minimizing the bias present in some experimental methodologies, selecting a suitable experimental platform may assist quantification. Colony-forming units (CFU) are used to quantify conjugation by selecting markers (such antibiotic or heavy metal resistance, which often reside on plasmids) that may identify transconjugants from parental strains (Johnsen & Kroer, 2007; Neilson et al., 1994; Andrup & Andersen, 1999; Singh et al., 2013). By maintaining total separation amongst conjugation and selection, as previously mentioned, their effects on the conjugation process should be easily discernible if the antibiotic being tested is comparable to the one used for plating. Phenotypic readouts, in contrast, provide a benefit since higher throughput technologies, including plate readers as well as flow cytometry for fluorescence, may be employed (Sørensen et al., 2003). With the right reporters, single-cell and microfluidics analyses are made possible by quantification employing microscopy (Babić et al., 2008). In fact, utilizing fluorescently tagged donors, recipients, and transconjugants, a microfluidics platform was recently employed to measure conjugation dynamics (Lopatkin et al., 2016). However, the detector must be capable of picking up events that happen at low frequencies. In some systems, the conjugation efficiency may alternatively be determined via nucleic acid measures, such as qPCR (Wan et al., 2011); this method of quantification does not need transconjugant enrichment (for example, through selection), and it may be able to avoid biases brought on by growth.

Experiments should be planned to provide comparable outcomes regardless of the platform. As an illustration, the impact of antibiotics on various systems might introduce additional

compounding variables, such as variations in the antibiotic sensitivity of donors and receivers. The minimal inhibitory concentration (MIC) or IC50 value of a medication, as well as whether the antibiotic examined correlates to the resistance conferred, must be understood in relation to the concentrations employed in each specific investigation. When comparing efficiency, it is important to take into account the fact that various systems may need varying timescales.

Antibiotic Resistance Transmission via Transformation

HGT is the transfer of genetic information from one bacteria to another bacteria within the same generation. The active uptake of free DNA by bacterial cells and the heritable inclusion of its genetic material constitute natural genetic change. The cellular processes involved in transformation have been extensively studied through in vitro experimentation with a few transformable species since Griffith's famous discovery of transformation in *Streptococcus pneumoniae* in 1928 and the demonstration of DNA as the transforming principle by Avery and coworkers in 1944. Transformation maybe a potent horizontal gene transfer method in naturally occurring bacterial populations, although this idea has only recently come to light (LORENZ & WACKERNAGEL, 1994). DNA internalization and chromosomal integration are required for natural bacterial transformation, which has been observed in about 80 species so far, requires DNA internalization and chromosomal integration. Recent research has shown that phylogenetically distinct animals differ in the inciting cues and regulatory processes involved, whereas they share conserved uptake and processing proteins.

There are three types of gene transfer systems, i.e., conjugation, transformation, and transduction. In the previous conjugation method, we saw that in order to transfer genetic information, bacteria must be physically attached to each other. In contrast, the transformation process is a genetic recombination method in which lysed and degraded bacteria are enter into the competent recipient bacterium and incorporate the piece of DNA into the recipient bacterium. That means, the transformation process directly uptakes the foreign DNA from the surrounding environment. Again, normal bacteria are not able to uptake the foreign DNA particles directly via the membrane. For that reason, the competent cell can easily take the foreign DNA particle. During the growth of bacteria, they pass certain phase like the exponential phase, which is a

competent phase. In this phase, the cell membrane is a permeable surface that allows the liquid-type materials in or out. Some examples of naturally competent cells are *Neisseria gonorrhoeae, Neisseria meningitides, Hemophilus influenzae, Legionella pneomophila, Streptococcus pneumonia, and Helicobacter pyloria* (Libretexts Biology & Kaiser, 2022).

Kinetics of Transformation Reaction

During transformation, the competent cell directly uptakes the foreign DNA from the surrounding environment. In the surrounding environment presents billions of bacteria which are created by manmade activities, animals, industries and so on (Prussin & Marr, 2015). Most of them are pathogenic or nonpathogenic. Pathogenic are those bacteria that are disease-causing, whereas nonpathogenic bacteria are not the cause of the disease. Moreover, these pathogenic bacteria may be AMR, meaning these bacteria can fight against medicine (World Health Organisation, 2020). So, these existent bacteria are broken down every day, naturally or unnaturally, via different factors like lack of oxygen, enzymes, detergents, and so on. After being lysed, the genetic materials are released into the environment. After that, the lysed DNA will bind with the cell wall of the recipient bacterium. Here, one DNA fragment of foreign DNA will be hydrolyzed by the envelope exonulease of the recipient cell. Therefore, only one DNA strand will enter the recipient cell. That means a linear DNA fragment entered. Once the fragment is entered, it will find the homologous region with the recipient cell after determining that it will be integrated into the host chromosome. Thus, if that happens, it will be a successful transformation. If the entered fragment does not find a homologous region in the recipient cell, it will degrade inside the cell and is referred to as an unsuccessful transformation. For example, a plasmid uptakes a green fluorescent protein gene from the surrounding environment and it turns into a green fluorescent bacterium.

Fragments of DNA from
the dead bacterium.

25
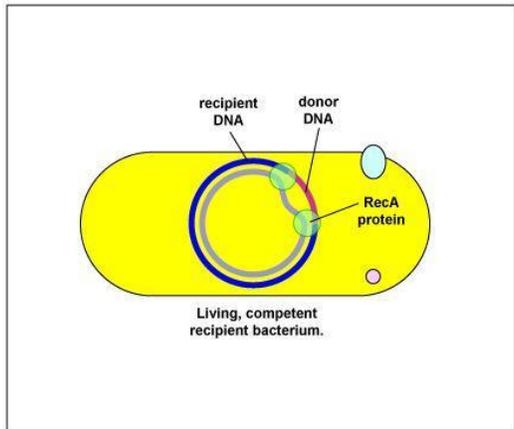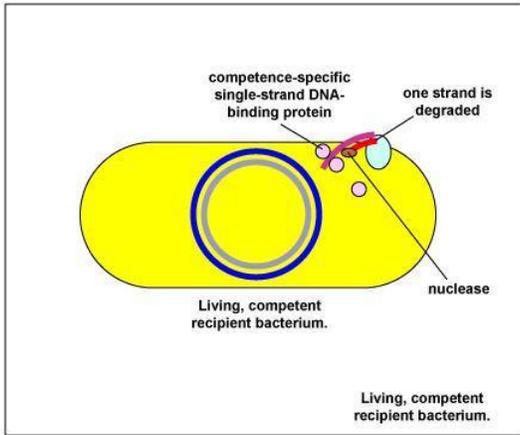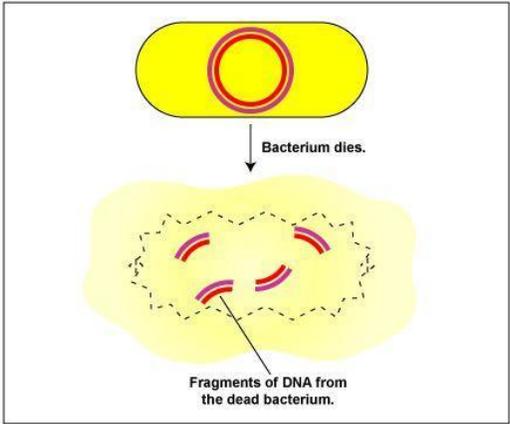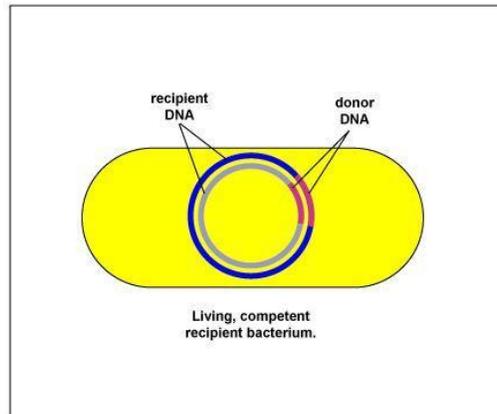
Figure 4: Flowchart of the overall transformation process. In the very first stage donor bacteria must lysed and released the genetic material in the environment. In the second step, the lysed DNA around 10 genes long band bind with the living recipient competent bacterium cell wall to transformation. In the third step, one DNA fragment of foreign DNA will degrade by envelope exonuclease by the recipient cell and another DNA fragment will entered into the recipient. Here, to prevent from being degrade in the cytoplasm the foreign DNA bind with the Competence-specific single-stranded DNA-binding proteins. In the fourth step, exchange a fragment of genetic information to promote RecA proteins between a donor foreign DNA and recipient bacterium. And the foreign DNA is incorporated into the recipient chromosome as can be seen in the picture.

The fifth step shows picture of successfully complete transformation and the flowchart pictures are taken from (Libretexts Biology & Kaiser, 2022).

In-vitro Process of Transformation

Till now, it has been described how the transformation occurs in the environment naturally. Additionally, the transformation process can also be performed in vitro, meaning in the lab setup. This experiment is done in the laboratory to produce multiple copies of our targeted gene via plasmid. Therefore, first insert the targeted gene into the circular DNA, which is called a plasmid. In this process, restriction enzymes and DNA ligase are used, which help in ligation .To perform this experiment first, our targeted DNA will be designed. Here, for cutting, the designed foreign DNA fragment will be cut by using a restriction enzyme. After that, the recipient bacteria must be competent phage. As all bacteria are not naturally competent to uptake foreign DNA, they must be converted to competence via chemical manipulation in the laboratory. Different ways to convert competent cells, e.g., treating cells with solutions of CaCl2 or chlorides of other elements such as Mg, Ba, Rb, Sr, and mixtures of them; (ii) treating cells with chelating agents (e.g., EDTA); (iii) treating cells with enzymes (muraminidases or peptidases), resulting in the formation of spheroplasts or protoplasts; (iv) fusing cells or protoplasts with DNA (bio listic transformation). Here, we commonly use calcium chloride, which helps to permeabilize the cell membrane so that the bacteria can uptake our targeted foreign DNA (LORENZ & WACKERNAGEL, 1994, P565). Now, use the same restriction enzyme to open the plasmid gene and it will be ready to accept the piece of new DNA (Cecchetelli, 2019). Finally, the incorporated foreign DNA is ligated with the recipient plasmid with the help of a ligase enzyme. Once the experiment is successfully done, it will be cultured in the lab and make a multiple number of copies (Science Learning Hub, 2014). Furthermore, because the foreign DNA is circular, the degradation of double-stranded DNA is less than that of single-stranded DNA, as previously mentioned.Also, plasmids can replicate independently, and they can replicate multiple numbers of genes inside the cell and produce a good amount of desired product.
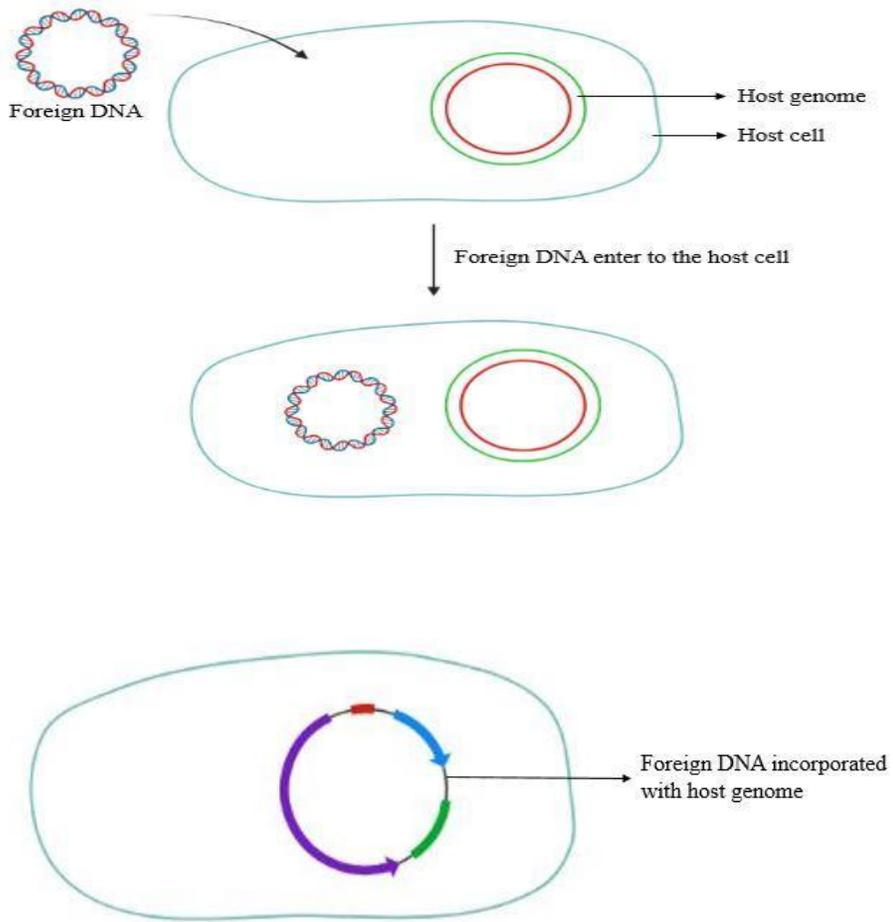
Figure 5: The in-vitro transformation process. Pictures are made by biorender.

Transfer Rate End-point Equation

The end-point equation is used to determine the transfer rate from experimental data.

$$\gamma = 1 \Delta t\ (V0 - R0)\ (\ ln\ (V0 - R0 + R2R1)\ -\ ln\ (V0R0))$$

Here, Δt = time interval of transfer; V0 & R0 = initial concentrations of vector and recipient cells; R1 = final concentration of recipient cells and γ = transfer rate per recipient cell concentration and vector concentration. (von Wintersdorff et al., 2016; Frontiers in Microbiology et al., 2018)

For example, we used the end-point equation to calculate the value of γ transformation with data taken from the published transformation study (Lu et al., 2015).). Here, we used average DNA and mass DNA fragments of about 30 kb and converted between count and DNA concentration. The published paper reported a DNA concentration of 2.5 µg/mL at one data point, which translates to a V0 of 8.33×10^{11} fragments/mL and an artificially induced recipient cells' competence rate is about 0.2. As the same data point, the reported recipient concentration of 10^7 cells/mL translates to an R0 of 2×10^6 cells/mL. So, at that point, the value of R1 is calculated as 1.9998×10^6 from the stated transformation frequency. According to these three values Δt of 30 min, so, $\gamma = 4.00 \times 10^{-17}$ mL DNA fragment-1 min-1. Again, its repeat for each amount reported in the paper generates a mean of $\gamma = 4.35 \times 10^{-17}$ mL DNA fragment-1 min-1. Now, to get the final γ transformation, the receiver competence rate and the ratio of free DNA to donor cells must be scaled into this rate constant. Although different bacterial species have different competence rates in nature, this model uses a suitable competence rate of 0.01 (von Wintersdorff et al., 2016; Lorenz & Wackernagel, 1994). So, this competence rate is directly proportional to the γ transformation. Based on estimations of 10^6 cells/mL and 0.01 g/mL of DNA in ocean water, the ratio of vector concentration to donor cell concentration employed translates to a ratio of 333 DNA pieces per donor cell assuming an average fragment size of 30 kb (Jiang & Paul, 1998; Lorenz & Wackernagel, 1994; Lu et al., 2015; von Wintersdorff et al., 2016). From these two factors, the final γ transformation is approximately 10^{-16} mL cell-1 min-1. Only 1% of bacterial species can naturally change. Since these species are widely scattered among the taxa, it is appropriate to choose each species' transformability at random with a probability of 0.01 to mimic natural transformation (Jhontson et al., 2014, Thomas & Nielsen, 2005).

Transformation Method of Gram-positive and Gram-negative Bacteria

Gram positives transformation method is based on the agitation of bacterial protoplasts with glass beads in the presence of DNA and polyethylene glycol (Rattanachaikunsopon & Phumkhachorn, 2009). One of the main obstacles to genetic studies of Gram-positive bacteria is the challenge of delivering DNA into the cells of Gram-positive bacteria. Their cell walls' substantial peptidoglycan coating is seen as a potential impediment to DNA uptake. These organisms cannot be transformed spontaneously, but they can be altered using specific transformation techniques like protoplast transformation and electroporation. To produce protoplasts by enzymatically removing the cell wall, a process known as protoplast transformation was created. Polyethylene glycol makes it easier for protoplasts to absorb DNA. For several Gram-positive bacteria, it was created (Morelli et al., 1987). This approach is infrequently utilized due to its low and extremely variable efficiency and time-consuming methodology. For Gram-positive bacteria, electroporation is now the most popular transformation technique. To stimulate the creation of transitory holes in cell walls and membranes, a high voltage electric pulse with a brief duration is applied. The pores may allow DNA from the surrounding environment to enter under the right circumstances. DNA was successfully inserted into numerous types of Gram-positive bacteria using this technique. Although electroporation can be used to change bacteria with high effectiveness, due to the demand for expensive and specialized equipment, it cannot be carried out by small and underequipped laboratories. Gram-positive bacteria were used as the recipients for pGK12, an erythromycin resistance gene-carrying 4.4 kb *E. coli/Lactococcus* shuttle vector, to achieve glass bead transformation. *Enterococcus faecalis TISTR 927, Lactobacillus casei ATCC 393, Lactococcus lactis DSM 20481, Leuconostoc dextranicum ATCC 19255, Listeria innocua DSM 20649, Staphylococcus aureus ATCC 25923, and Streptococcus pneumoniae ATCC 10015* were the bacteria employed in the transformation studies (Rattanachaikunsopon & Phumkhachorn, 2009).

Additionally, in Gram-negative by using FT-IR analysis of the frequency shifts of the acyl chain methylene symmetric stretching band as a monitor, temperature-induced order/disorder transition profiles were discovered in the membranes of intact Gram-negative bacterial cells. Different

transition profiles were produced by cells grown at various temperatures. But at each growth temperature, the virtually same frequency readings showed that the bacterial membranes were in a very similar "state of order." A GC analysis of the fatty acid composition of entire cells was used to supplement the FT-IR findings. The in vivo FT-IR data provided clear proof that bacterial membranes can change their "state of order" and "fluidity" to a range of growth temperatures (Schultz & Naumann, 1991).

Five Models Explaining Bacterial Uptake of DNA from the Environment

According to the first hypothesis, DNA is ingested by the bacterial cell and used as a source of nutrients (Finkel & Kolter, 2001). Studies demonstrating that in nutrient-limited circumstances, some bacterial strains are capable of assimilating DNA corroborate this concept (Redfield, 1993). By switching the cultures from a rich medium to a starving media, many bacterial species, including *Gallibacterium anatis, Haemophilus influenza, and Aeromonas salmonicida26*, are brought to competence in vitro (Huddleston, 2014). More particular, purine deprivation causes H. influenzae to become competent.

The second hypothesis postulates that natural transformation arose so that altered DNA molecules might function as templates for DNA repair, explaining the evolutionary roots of competence development and natural transformation (Huddleston, 2014). When DNA is applied to *Bacillus subtilis* cells that have been exposed to ultraviolet light after damage has occurred, the cells' survival rate is higher than when DNA is applied before damage. It is likely that the additional DNA acts as a template for re-combinational repair (Michod et al., 1998). When exposed to the DNA-damaging chemical *mitomycin C, Streptococcus pneumoniae* can develop competence, more specifically the com regulon.

A third hypothesis holds that natural processes evolved to enable cells to acquire novel genetic information on purpose in order to broaden their genetic variety and better withstand natural selection. But according to research on the transformability of *Acinetobacter baylyi*, competent and incompetent strains both acclimated to laboratory circumstances at the same rate (Bacher et al., 2006). Competence did not bring about any observable benefits, and the competent lineages

even evolved a lower level of transformability in lab settings. In their recently published complementary model, Engelstädter and Moradigaravand hypothesized that competence evolved as a result of bacterial cells' ability to take up DNA from their environment that has endured for an infinite amount of time and incorporate genes into their genomes in order to return to a previous genetic state (Huddleston, 2014).

The fourth hypothesis proposes that periodic stressful situations, which may unintentionally favor non-growing competent cells, are responsible for the evolution and maintenance of natural transformation. Competent cell populations, like Bacillus subtilis, need many hours to grow. This state is regarded as "per-sistering." The per-sister state was initially identified in 1944 in penicillin-treated Staphylococcus cells that survived. These cells survived not because they were mutants with different antibiotic targets, but rather because they were not growing and unaffected by the antibiotic (Huddleston, 2014). Instead of being resistant to antibiotics, these cells are now thought to be tolerant of them. When the cell population is exposed to stressful situations that kill growing cells more quickly than per-sister cells that are not expanding, this process of episodic selection of per-sister cells takes place. If these per-sister cells are also competent, as some of them are, then the stress also indirectly selects for natural transformation ability. The use of antibiotics (penicillin-G) as episodic selection in populations of B. subtilis, where capable cells outlived non-competent mutants, has been used to support this idea (Huddleston, 2014; Johnsen et al., 2009).

The final model that Natural transformation is a byproduct of the twitching motility and cell adhesion activities of type IV pili (Huddleston, 2014). It is well known that Type IV pili absorb free DNA during natural transformation. The tip of the type IV pilus, PilC, may unintentionally bind DNA as the cells move over surfaces using their twitching motility. The PilQ pore in the outer membrane is blocked by bound DNA as the pilus retracts, and it eventually passes through after one strand is broken down by nucleases. According to this hypothesis, environmental stress activates the SOS response, a DNA repair system that is prone to errors, which causes the development of type IV pili, allowing the cell to move by twitching motility to a more advantageous environment. The active type IV pili result in the binding and uptake of free DNA by the cell (Huddleston, 2014; Bakkali, 2013). The capacity to naturally convert is accidental in this scenario, but it can be useful if the cell can use the new DNA as a source of nutrients or as a

model for genome repair. The recipient cell's genotype changes as a result of transformation, regardless of the biological cause for the uptake. Microorganisms frequently undergo transformation, which has a long-lasting effect on bacterial genomes that is challenging, if not impossible, to measure (Huddleston, 2014; Beiko et al., 2005).

Effects of Antimicrobial Resistance in Transformation

Antibiotic-resistant illnesses are seen as a serious worldwide health concern. It is thought that the era of antibiotic therapy's greatest success is coming to an end and that we are on the verge of returning to a time before antibiotics, when there were few effective therapies for infectious diseases brought on by bacteria. Both in terms of the economic costs to society and the morbidity and mortality of those affected, these resistant illnesses are expensive. Infections with third-generation cephalosporin-resistant Escherichia coli and methicillin-resistant Staphylococcus aureus alone cause hundreds of fatalities, countless additional infections, and millions of dollars' worth of medical expenses each year (de Kraker et al., 2011). Pharmaceutical companies have drastically reduced the amount of time they spend developing antibiotics, now making up only approximately 0.2% of all new medication development, even though infectious diseases are still the largest cause of death globally. Treatment choices are becoming more difficult as antibiotic resistance particularly that of bacteria resistant to multiple drugs, continues to evolve (Huddleston, 2014). Moreover, a favorable environment for the emergence and dissemination of antibiotic resistance genes in bacterial populations is provided by the human gastrointestinal tract. The presence of many cells is one of these factors.

For example, a kind of bacteria that often picks up additional DNA Neisseria is a spontaneous transformation involving horizontal gene exchange. They're among the most prevalent and resilient *Neisseria gonorrhoeae* is a species of *Neisseria*. This species is capable at all phases of development and ingests DNA from contains a 10 kb DNA uptake sequence peculiar to the genus (GCCGTCTGAA) (Hamilton & Dillard, 2006). In *N. gonorrhoeae,* this sequence arises around once per 1000 bp. Neisseria autolysis, Type IV secretion, and other less well-known secretion techniques can all release donor DNA into the extracellular environment. *Neisseria* transformation is obviously significant and may be conserved due to its involvement in producing variable surface antigens that help evade the host immune response. Multiple

resistance determinants have been successfully transmitted among N. gonorrhoeae by frequent transformation. The significant prevalence of drug resistance to fluoroquinolones and β-lactams has seriously hampered their ability to effectively treat *N. gonorrhoeae* infections. Amino acid changes in PBPs are the mechanism by which N. gonorrhoeae is resistant to β-lactam antibiotics. One study discovered a mosaic PBP-2 protein in *β-lactamresistant N. gonorrhoeae* that differed from PBP-2 in susceptible bacteria by 60 amino acid changes and was thought to be a contributing factor to *β-lactam* resistance. By transforming the DNA of resistant, non-pathogenic, commensal *Neisseria, Mosaic PBP-2* was able to acquire the mutations required for the conferral of resistance (Spratt et al., 1992). Although they are not pathogenic, the species *N. flavescens and N. cinerea* are naturally more resistant to *β -lactams* than *N. gonorrhoeae*. As a result, they have spread the infection and made it more difficult to treat N. gonorrhoeae infections. For the purpose of researching interspecies transformational events, the evolution of resistance genes in the *Streptococcus and Neisseria* species has made for a useful model. Without the strong selection that antimicrobials subject bacteria to and the careful attention paid to emerging resistance phenotypes, it is doubtful that transformation in *S. pyogenes* would have been detectable (Ojha et al., 2021). Without the transfer of antimicrobial-resistance determinants, it is also doubtful that the transfer of genes from commensal to pathogenic species of *Neisseria* would be as easily discovered. These instances of the transformational transfer of resistance determinants highlight the value of utilizing antimicrobial resistance as a model to investigate fundamental microbial mechanisms and population genetics (Ojha et al., 2021; Barlow, 2009).

Another example, a crucial mechanism for the propagation of resistance among the *streptococci* is transformation, or the uptake and integration of DNA from the environment. The pathogen *Streptococcus pneumoniae* is significant and widespread. It also causes meningitis, severe sinusitis, otitis media, septic arthritis, and other diseases in addition to respiratory illnesses (López, 2006). *S. pneumoniae* frequently gains fresh DNA through natural evolution. The competence factors underlying *S. pneumoniae's* inherent competence have been thoroughly investigated and discussed. Penicillin resistance is common in *S. pneumoniae*, and it appears to be connected to how frequently *streptococci* change (Ojha et al., 2021; Desai & Morrison, 2006). The frequent occurrence of transformation within *S. pneumoniae* populations may be a factor in the pathogen's high level of penicillin resistance. Another extremely common infection found around the world is *Streptococcus pyogenes*. Although it can cause more serious infections as

well, it most usually colonizes in the nasopharynx and produces superficial infections of the epithelium (Ojha et al., 2021; Passàli et al., 2007). *S. pneumoniae* is frequently resistant to penicillin, although *S. pyogenes* is generally thought to be penicillin-susceptible despite frequent exposure. It doesn't happen often for S. pyogenes to transform. One theory for *S. pyogenes'* continued vulnerability to penicillin is because the organism rarely transforms. Strong selection for fluoroquinolone resistance, however, has uncovered elements of transformation in *S. pyogenes* that were not previously known. Fluoroquinolones have become widely utilized in recent years to treat infections caused by streptococci (Pletz et al., 2006). Fluoroquinolone resistance is caused by mutations in the parC component of topoisomerase IV and the gyrA gene, which codes for gyrase. Fluoroquinolone resistance-causing substitutions tend to happen in specific "hotspots." The "quinolone resistance determining region" (QRDR) is what is known as this region and it is conserved in both Gram-positive and Gram-negative bacteria. Fluoroquinolone resistance in S. pyogenes is uncommon, but it has been observed (Ojha et al., 2021; Yan et al., 2000). Interestingly, HGT of the QRDR of parC was the best explanation for resistance in some strains, according to the analysis of the resistant strains, which revealed that point mutations in parC were the cause of resistance in some strains. Even more surprisingly, the sequence of the recombined DNA segments revealed that the segment causing fluoroquinolone resistance had originated from the absorption of DNA from a different species, *S. dysgalactiae* (Ojha et al., 2021; Pletz et al., 2006). As a result, transformation among *S. pyogenes* might happen more frequently than previously thought. The data also suggest that *S. pyogenes* is capable of consuming DNA from many species. These findings show that using antimicrobial resistance as a model for researching HGT and evolution in bacteria allows for the detection of unusual events (Ojha et al., 2021; Barlow, 2009).

Antibiotic Resistance Transmission via Transduction

Transduction is a method through which organisms acquire DNA.whereby non-viral DNA can be transmitted by infectious or non-infectious virus particles from an infected host bacteria to a new host. When the phage particle forms, host DNA is inadvertently packaged into the empty phage head. Infectious phage particles that are liberated from lysed host cells have the ability to bind to fresh host cells and deliver the DNA contained in the capsid. The recipient's genome may incorporate the injected bacterial DNA. Although the majority of bacteriophages only infect a small number of hosts, this method of gene transfer has the benefit that transducing phages may be rather tenacious in their environment, DNA in the transducing phage particles is protected, and there is cell-cell interaction. Recent research on the number of bacteriophages in various environmental settings and data from bacterial genome sequences are the major sources of evidence for the significance of transduction as an HGT mechanism under environmental circumstances. By using electron or epifluorescence microscopy to count bacteria, bacteriophages were shown to be around ten times more numerous than bacteria in fresh or marine water samples. Directly counting bacteriophages in soil is clearly more difficult, and only lately have studies found that soil viruses are also abundant. (HEUER, 2007).(Fard, 2011).

Transduction is one instance where bacteria have been hypothesized to benefit from phages. Transduction is regarded as the primary factor causing the dissemination of antibiotic resistance genes and the pathogen's success.As a result of an abnormal prophage excision, DNA bordering the prophage attachment site (attB) is transferred during specialized transduction. Recently found lateral transduction results in extremely effective packaging of bacterial DNA several hundred kilobases downstream of the integration site by late excision and in situ replication of an integrated prophage (Institute of Medicine et al., 2011; Salom, 2019). Transduction has historically been undervalued as a key method of horizontal gene transfer in naturally occurring ecosystems. Up to 50% to 60% of bacteriophages may include functional bacterial genes of all sorts, according to a metagenomic investigation of viromes. These particles may potentially operate as a conduit for the transfer of genes between bacteria.When a bacteriophage replicates in one bacterial cell before moving on to another, the process known as transduction takes place. The donor bacterial cell's genome is packed into the phage head and transferred to the recipient bacterial cell. It is believed that transduction is an unevolved process that results from the errors

made during the excision of bacteriophage DNA from the donor genome. (Huddleston, 2014).Microbial organisms can acquire additional genetic material from sources other than their clonal ancestry thanks to (HGT). Microbes may sample and share a sizable gene pool through HGT, which may contain features that are advantageous in their immediate context. For instance, horizontal acquisition of antibiotic resistance genes (ARG) permits diversity of genomes and generates a possibility for fast fitness gains when bacteria are subjected to strong selective pressures, such as the presence of antimicrobials. In fact, HGT can produce the genes needed for survival more quickly than spontaneous mutations.It is recognised that transduction, particularly between individuals of the same species, may play a role in the spread of ARGs. When viral particles transfer bacterial genes, transduction takes place. Bacteriological DNA may inadvertently get wrapped in a bacteriophage capsid during bacteriophage infection. A recipient cell may connect to a capsid harboring bacterial DNA and receive the foreign DNA with no problems. Transduction has taken place if the bacterial DNA has been recombined into the receiving cell's genome. (Lerminiaux, 2018).

Classification of Transduction

Transduction can be divided into broad categories. Specialized transduction takes place when only bacterial DNA close to a temperate bacteriophage's attachment site, such as that found in E. coli, is unintentionally packed into the bacteriophage and then transported to a recipient cell.When any gene from a host gets arbitrarily packed with viral DNA into a bacteriophage head, as happens in Salmonella P22, and then transmitted to a new recipient, this is known as generalized transduction. As bacteriophages can remain in the environment for variable length of time, the donor strain and the receiver strain do not necessarily need to be near to one another in terms of time or space. Almost every DNA sequence that can be identified in the bacterial genome may be transferred, including antibiotic resistance. Chromosome sequences and mobile genetic elements like plasmids, transposons, and insertion elements are included in this. (Huddleston, 2014).

Generalized transduction was a potent genetic tool when it was first discovered, revolutionizing microbial genetics at the time. It was also discovered early on that the transductants, or cells that

receive bacterial DNA, frequently contain a copy of the phage in their genome, transforming them into lysogens. It was not obvious from this whether the bacterial DNA was transmitted separately or with active phage particles. Studies later showed that the viral particles causing generalized transduction contain bacterial DNA and are not phage functional (Institute of Medicine et al., 2011). Based on these discoveries, transduction has long been thought to result from flaws in the phage DNA packing mechanism that allow bacterial DNA to be packed instead of phage DNA.In keeping with our ongoing research on the function of transduction in phage-host interactions, we now suggest that infection of a bacterial cell by a temperate phage and a transducing particle can improve the fitness of both by enabling the host cell to develop adaptable genes that benefit both the prophage and the phage. This establishes a clear link between transduction and phage fitness, which may help to explain why some phages have high transduction rates. We demonstrate that, under a specific set of circumstances, generalized transduction offers the phage bet-hedging opportunities that increase both its own and the hosting cells' chances of survival in shifting environments (Institute of Medicine et al., 2011). Either the lytic cycle or the lysogenic cycle is used for transduction. The phage chromosome can be incorporated into the bacterial chromosome and remain inactive for hundreds of generations if the lysogenic cycle is used. The lytic cycle, which results in cell lysis and the release of phage particles, is started if the lysogen is triggered (for instance, by UV radiation). This excises the phage genome from the bacterial chromosome. New phage particles are produced throughout the lytic cycle and released when the host is lysed (Carpa,2010).The advantages of generalized transduction to the phage call into doubt the notion that it is simply a packaging fault. Naturally occurring phages of both Gram-positive and Gram-negative bacteria exhibit a wide range of transduction efficiency, with many of them being unable to do so (Institute of Medicine et al., 2011) . On the side of the host, bacteria gain from the protection that prophages bestow upon other phages in their family as well as from products encoded by prophages, such as virulence factors. Once developed, the transducing prophages may use autotransduction to pick up genes from other cells. In light of the findings presented here, which show that lysogeny and the acquisition of features like antibiotic resistance facilitate phage survival, transduction appears to be a cooperative technique used by some phages to ensure the life of the host. Defense mechanisms based on phage absorption prevention will stop DNA from transducing particles from entering, and restriction-modification mechanisms will stop transfer if the DNA comes

from a strain missing the mechanism. In certain circumstances, the acquisition of new features may occur by means of different mobile genetic components, including conjugative plasmids, or by spontaneous transformation (Institute of Medicine et al., 2011) .

The second transduction process to be uncovered was specialized transduction, which was found in the coliphage. Specialized transduction is only capable of transferring particular gene sets, as opposed to the generalized process that can package and transmit any type of bacterial DNA. When viral and bacterial host DNA are encapsidated as a hybrid molecule, specialized transducing particles are produced. The process that creates these specific transducing particles is based on the conventional phage model, in which a segment of viral DNA is joined to an adjacent length of DNA from the host bacterial chromosome in the excised molecule by aberrant prophage excision events.Once episomal, the hybrid molecule replicates similarly to a typical viral genome. Specialized transduction is thought to contribute little to overall phage-mediated gene transfer since incorrect excision is uncommon and the amount of bacterial DNA that may be transported is constrained. It's interesting to note that while specialized transduction is typically thought to be the realm of cos-type phages, pac-type phages may also use it.(Chiang, 2019).

Lateral transduction, the third transduction mechanism, was recently found in the Staphylococcus aureus temperate phages.Contrary to its forerunners, lateral transduction does not seem to be the outcome of a mistaken phage process. Instead, it appears to be an organic component of the phage life cycle. The fact that the staphylococcal prophages excise late in their life cycle rather than following a conventional lytic program is crucial in this situation. This leads to a mechanism of transduction where bacterial chromosomal DNA is transferred at frequencies that are at least 1000 times higher than those previously recorded.Prophages are believed to excise and circularize quickly upon lysogenic induction, in accordance with the usual excision-replication-packaging process. Because DNA packaging before excision would cleave the viral genome in two, it is believed that the order in which these activities take place is crucial. The bacterial chromosome has long segments that may reach lengths of several hundred kilobases. These segments are packed and transported at frequency that are unheard of for the majority of gene transfer processes. An innovative interpretation of the idea of mobile genetic elements that is determined by genomic coordinates, rather than by the DNA components

themselves, is that regions of the bacterial chromosome become "hypermobile platforms" of gene transfer. Last but not least, in situ theta replication generates several integrated genomes to enable simultaneous in situ DNA packaging and phage maturation in order to counteract the anticipated disastrous effects (breaking the viral genome in two) of in situ DNA packing. So, with the creation of wild-type phages, staphylococcal phages naturally create exceptionally large titers of lateral transducing particles (Chiang, 2019).

Transduction Related to Lysogenization

Lysogeny is quite prevalent among the microorganisms under investigation in the current study, with nearly all clinical strains being lysogens. Only a small percentage of strains include CRISPR-Cas systems, and within the lineages created by the barriers of restriction-modification systems, interchange of phages and DNA happens regularly. Cooperation between these bacteria and temperate, perhaps transducing phages may be a survival tactic that not only permits the interchange of mobile genetic elements and resistance genes but also may potentially have an impact on how the bacterial genome evolves within lineages.It was discovered that the amount of bacterial DNA carried in phage lysates supports the transduction of unlinked chromosomal and plasmid markers and that each milliliter of phage lysate will contain roughly 20,000 copies of the bacterial genome when we looked at the bacterial content of a phage lysate from the staphylococcal phage 11 (Institute of Medicine et al., 2011) . We previously observed that a lysogenic strain releases a significant amount of 11 spontaneously, and we hypothesized that this might be significant for the acquisition of traits by auto-transduction, in which released phage spread on susceptible bacteria in the environment and returning transducing particles provide resistance to, for example, antibiotics. Since they may be released as part of lateral transduction, phage particles released spontaneously from or by stimulation of lysogens are expected to carry even higher percentages of transducing particles (Institute of Medicine et al., 2011). As demonstrated above, these transducing particles will be able to transfer genetic data across cells that are infected with the same temperate phage, demonstrating that lysogeny with a transducing phage permits either universal or lateral transmission of genetic information. In conclusion, our findings demonstrate that generalized transduction is a mutualistic characteristic that supports

both phage and lysogen survival, and crucially, that transduction is advantageous to temperate phages.(Institute of Medicine et al., 2011; Salom, 2019).

Transduction in Clinical Settings

There are less direct evidence exists for now that transduction takes place in hospitals. In the lab, it was discovered that bacteriophages obtained from MRSA infections contracted in hospitals easily transferred ARGs to vulnerable strains. Transduction can significantly contribute to the establishment and persistence of AR in clinically relevant S. aureus due to the fact that bacteriophage-mediated transfer of AR can take place in lab settings. Multiple ARGs, including ESBL genes, have been seen to be transferred via transduction in Gram-negative bacteria from Pseudomonas hospital isolates to other Pseudomonas strains in the lab.Some Gram-negative microorganisms that produce ESBLs developed through transduction. (Lerminiaux, 2018).

Kinetics of Transduction Reaction

A bacterial virus (bacteriophage) transduces DNA from one cell to another during this process. There are two ways that viruses can spread host genes. In the first method, known as generalized transduction, the mature virion is packed with DNA taken from essentially any region of the host genome instead of the viral genome. In the second method,known as specialized transduction, DNA is directly incorporated into the viral genome, typically replacing part of the virus genes. Only specific temperate viruses cause this. (Karki, 2018).

Generalized transduction occurs when every donor DNA fragment from every chromosomal location has an equal opportunity to enter the transducing bacteriophage. Bacteriophage infects the donor cell and starts the lytic cycle. When a virus enters a bacterial cell, it takes over the host cell and starts to make viral components such the genome, enzymes, capsid, and fibers at the head, tail, and tail ends.After that, a viral enzyme fragments the DNA of the host cell. Sometimes donor DNA pieces from donors are integrated into the viral capsid during the assembly of virus components to create offspring viruses (bacteriophage head).Such an aberrant bacteriophage can introduce donor DNA into new bacteria when it infects a cell. Due to the fact that this donor DNA is not viral DNA, it does not multiply inside recipient bacteria but rather undergoes homologous recombination with recipient cell's chromosomal DNA to create recombinant

cells.The donor genes used in generalized transduction are not a component of a viral genome and are unable to replicate on their own. Without recombining with the recipient bacterial chromosome, the donor genes will be lost. Homologous recombination may also happen during specialized transduction. The donor bacterial DNA may, however, be integrated into the host chromosome during lysogeny since it is essentially a piece of a temperate phage.
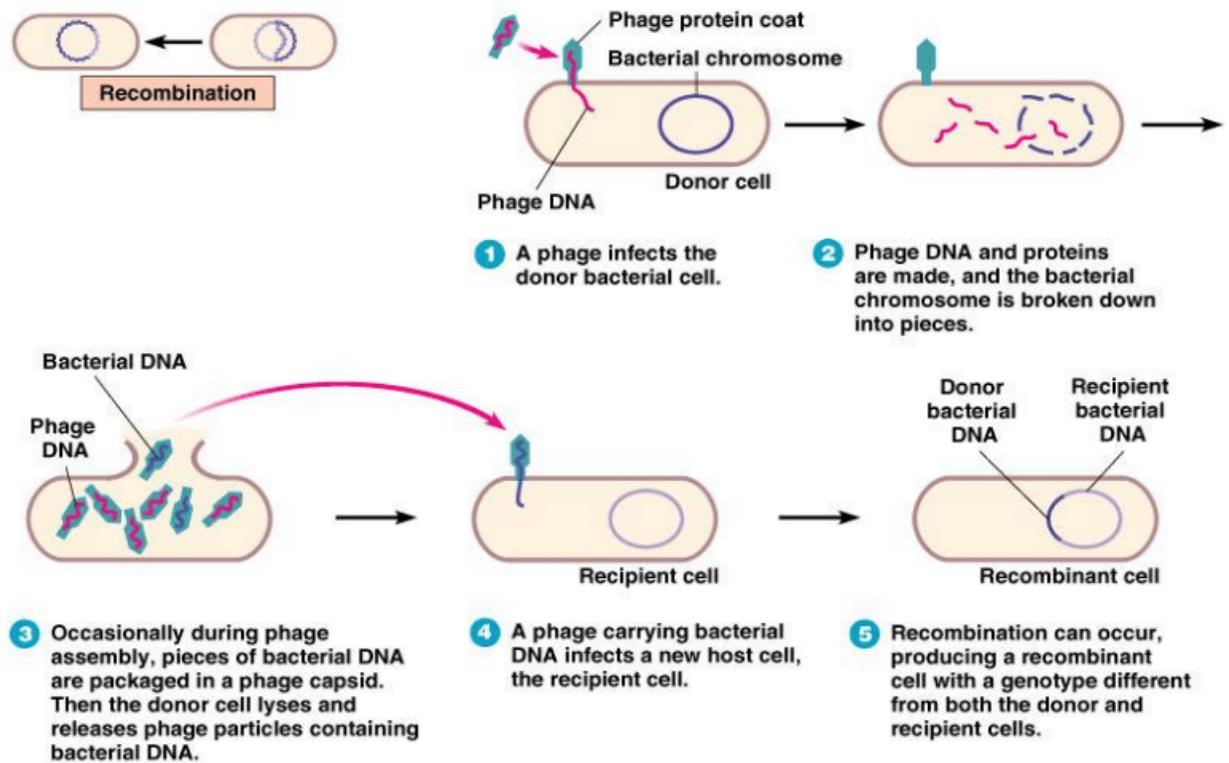

Figure 6: Generalized Transduction mechanism (Karki, 2018).

In specialized transduction, Bacteriophage only transmit a small number of limited genes (DNA fragments) from donor bacteria to recipient bacteria during specialized transduction. Only the temperate bacteriophage, which travels through the lysogenic cycle in the donor cell, carries out specialized transduction. Temperate bacteriophages first infect donor bacteria, after which their genomes integrate with the DNA of the host cell at a specific spot and go dormant, passing from generation to generation to daughter cells during cell division. Temperate phage is the name given to the bacteriophage that follows the lysogenic cycle. Such lysogenic cells induce viral genome from host cell genome and start the lytic cycle when exposed to particular stimuli, such

as certain chemicals or UV lamps.This phage genome occasionally carries a piece of bacterial DNA with it when it is induced from donor.(Karki, 2018).

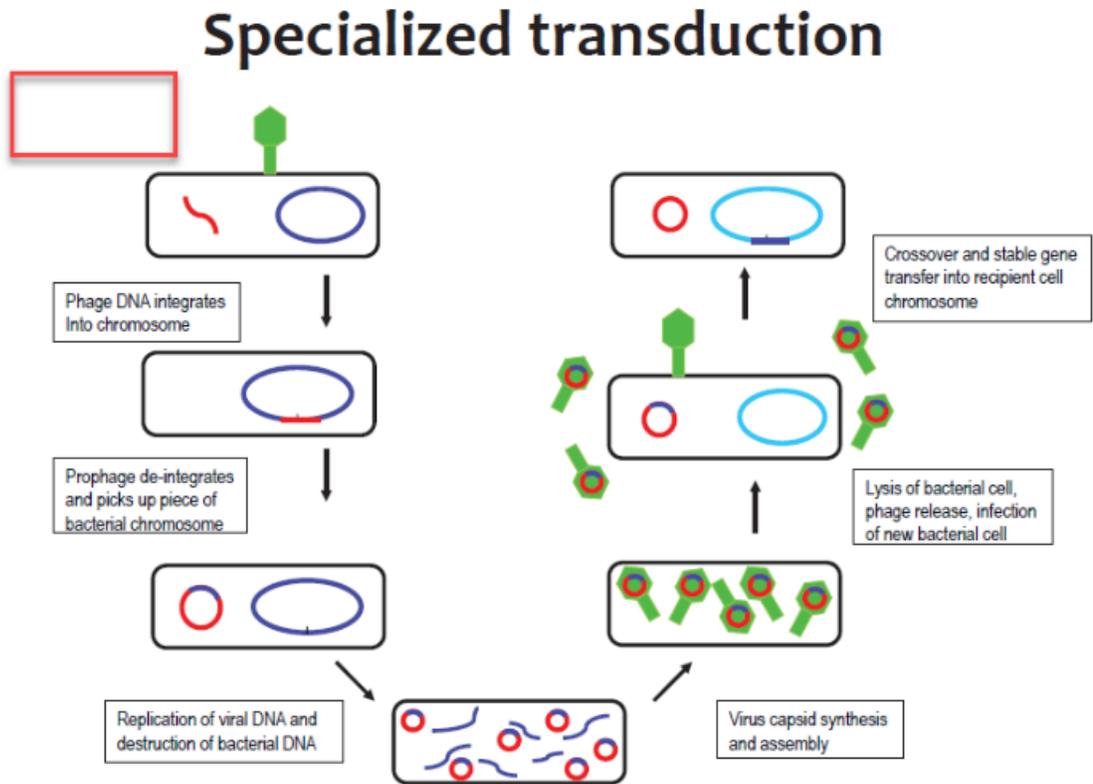
Figure 7: Specialized transduction mechanism (Karki, 2018).

Potential for Transduction of Soil Coliform Bacteria by Biosolids-derived phage

Three days were spent incubating aqueous solutions of soil bacteria with or without the addition of bacteriophages enriched from biosolids and antibiotics. As a typical group of bacteria within the overall soilborne microbiome, we decided to quantify coliform bacteria. The quantity of viable antibiotic-resistant coliform bacteria was unaffected by the addition of bacteriophages in the absence of additional antibiotics.When incubated with 1/100 of the cefoxitin breakpoint concentration, phage seemed to impart a minor reduction (less than 2-fold) in the number of cefoxitin-resistant coliforms. On the other hand, no therapy had an impact on the prevalence of ampicillin-resistant coliforms. Together, these findings imply that biosolids-derived bacteriophage enhanced the prevalence of coliform bacteria resistant to sulfamethazine or cefoxitin, but not in the absence of either antibiotic. Nevertheless, our findings demonstrate that, in the setting of an agricultural soil microbiome, subclinical amounts of particular antibiotics might enhance the horizontal transfer of resistance genes by phage into potential human pathogens.We use the word "transduction" loosely because the mechanism of gene transfer and whether it is generic or specialized have not been established, but we presume that the enrichment of antibiotic-resistant coliforms in the presence of bacteriophage is caused by transduction.Several lines of evidence lend credence to the idea that transduction was involved. First off, the impact only happened when enhanced bacteriophage was cultured with bacteria that were obtained from the soil. Furthermore, neither phage-only plating controls nor PCR tests of the purified bacteriophage preparations revealed any evidence of bacterial DNA, demonstrating that the bacteriophage enrichments were free of live coliform bacteria or bacterial DNA. Therefore, the increased resistance in the presence of enriched bacteriophage must be unique to the phage; the resistance genes might have come from bacteria in the biosolids from which the bacteriophage was enriched, or the bacteriophage might be encouraging the transfer of resistance genes within the soil bacterial community.Transduction is unquestionably a common process in many ecosystems and is known to be possible in soil. Muniesa came to the conclusion that phage-mediated horizontal transfer between intestinal bacteria or between intestinal and native bacteria in extraintestinal environments was likely based on factors like phage versus bacterial abundance, the number of phage in a transduction-competent state, and the physical conditions

of different environments. Furthermore, cultured murine intestinal microbiota can acquire antibiotic resistance from bacteriophages isolated from mouse feces treated with antibiotics. In-feed antibiotics cause prophage to appear in swine fecal microbiomes, and selection pressure is applied to horizontal gene transfer, including transduction.For instance, a research was shown at the frequency of HGT of ARGs in millions of microbial genomes discovered that selection pressure makes it such that the genes implicated in HGT events are 25 times more likely to become fixed in human-associated bacteria than in isolates from different environments. It is tempting to assume that transduction is often facilitated by antibiotic selection given our finding of likely transduction occurring only in the presence of selective pressure.It's likely that certain antibiotics favor transduction by various phage, each of which produces maximal transduction after a certain amount of time. It follows that it is probable that some ARGs are transduced more frequently than others. It is odd that, in the experiment, ampicillin selection did not result in transduction but cefoxitin selection did, given that beta-lactamases or mutations in the ampC promoter or attenuator might theoretically result in resistance to both antibiotics. One explanation is that ampicillin selection did not result in a strong increase in transduction like selection with cefoxitin did. As a result, various selection processes might have various potentiating impacts on the transduction frequency. It would be intriguing to see if transduction yields improved with different antibiotic choices. (Ross, 2015).

Roles of Transduction in Antibiotic Resistance

Another method of horizontal gene transfer that has influenced the evolution of bacterial genomes is transduction. Compared to spontaneous transformation and conjugation, bacteriophage-mediated antibiotic resistance gene transfer has less knowledge about it. However, it is now becoming more obvious how bacteriophages transfer antibiotic resistance genes. Bacteriophages are viruses that infect bacteria; they are said to be temperate if they integrate into the host genome and develop into prophages before the triggering of their lytic expansion by the environment.Evidence have showed that by the transfer of antibiotic resistance from Enterococcus gallinarum to Enterococcus faecalis during in vitro tests, transduction has only lately come to be thought of as having a key role in the transmission of antibiotic resistance

across potential pathogens. Ampicillin, chloramphenicol, and tetracycline resistance genes may be transduced in other in vitro studies using Salmonella transducing bacteriophages.(Huddleston, 2014). Antibiotics may be the driving force behind the transduction of ARGs since mice treated with antibiotics had more bacteriophages than mice not given antibiotics.Although at a slower pace than transduction of chromosomal resistance genes, bacteriophages were also capable of transferring a 5620 bp plasmid encoding a kanamycin resistance gene across Serratia and Kluyvera species.A Antibiotics may be the driving force behind the transduction of ARGs. In S. aureus, plasmid-borne ARG transmission bears the potential to be more effective than chromosomal methicillin resistance gene transduction. As a result, both chromosomal and plasmid-borne ARGs can spread by transduction, and antibiotics can speed up the rate of the process.(Lerminiaux, 2018).Tetracycline (tetM) and gentamicin (ant2-I) resistance were effectively transmitted across the same and distinct enterococcal species, indicating that enterococcal bacteriophages play a role in the transmission of antibiotic resistance genes in enterococci. The spectrum of medications that could be employed in the transduction studies was, however, constrained by the enterococcal isolates used in this work being resistant to a number of antibiotics.In the current investigation, gentamicin resistance was also conveyed as an antibiotic resistance. No isolates or transductants of enterococci exhibited gentamicin (1024 g ml1) resistance.(Wright, 2010).All of the bacteriophages used in the transduction experiment in the current investigation belonged to the Order Caudovirales (tailed bacteriophages), which is the most common order and family in nature. In this work, the transducing bacteriophages transmitted resistance genes between several species and were not species-specific. This is noteworthy because enteric bacteria including enterohaemorrhagic Escherichia coli, Salmonella, and Campylobacter are typed using bacteriophages, which are known to be very species-specific. (Fard, 2011).

Spread of Antibiotic Resistance

Antibiotic resistance raises major concerns for both the environment and human health worldwide. The danger of antibiotic resistance spreading is accelerated by the prevalence of antibiotic-resistant genes and microorganisms in the environment. One of the reasons the antibiotic resistance spread has grown so severe is because horizontal gene transfer, one of them, is a significant method in the transmission of antibiotic resistance genes. At the same time, the environment in which we live contains both host bacteria and free antibiotic resistance genes. They can influence not just horizontal gene transfer but also the spread and transmission of antibiotic resistance genes by migrating and gathering in various ways across environmental media (Huddleston, 2014; Lin et al., 2020). The impact of antibiotic resistance on the environment has emerged as one of the six most significant global environmental emerging challenges, according to the United Nations Environment Program's "Frontiers 2017" report. According to the paper, antimicrobial chemicals discharged into the environment by homes, hospitals, pharmaceutical systems, and agricultural runoff would directly encounter natural bacterial populations and increase the number of antibiotic-resistant bacteria (ARB). As a result, the spread of antibiotic resistance will cause a large number of antibiotic resistance genes (ARG) to accumulate in environmental bacteria, passing on resistance to pathogens that are frequently present in the environment or directly resulting in the creation of new pathogens, both of which pose a serious threat to human health (Ojha et al., 2021; Czekalski et al., 2012). Currently, antibiotic resistant is widely found in nature. Among the ARB, Methicillin-resistant Staphylococcus aureus (MRSA) is a prevalent bacterium which is known as "Super bacteria" since it is causative agent behind deadly infections and nearly immune to most antibiotics. MRSA is still a major cause of death worldwide (Gajdács, 2019).

The concentration of nine antibiotics ranged from ND (undetectable) to 2702 ng/g, ND to 449 lg/kg, according to a study on AR in surface water and sediments of cities in southern China. Corresponding ARG can also be found, such as quinolone resistance gene qnrA (~ 103 ARG/16S rRNA) and tetracycline resistance gene tetC ($\sim 10\text{-}2$ ARG/16S rRNA) (Ojha et al., 2021; Huang et al., 2019). According to research by Lord O'Neill that was released in the British media (O'Neill, 2014), by the year 2050, the number of fatalities caused by AR may total 10 million annually, or one death every three seconds, significantly exceeding the number of deaths brought

on by cancer. China has become more concerned about the proliferation of AR in recent years. China's government has released pertinent legislation and regulations. According to these strategies, bacterial resistance must be efficiently controlled at the national, social, and other levels in areas including hospitals, animal farms, environmental protection, and the general public. As a result, environmental research needs to comprehend and expose the concerns connected to AR and suggest priorities and measures (Ojha et al., 2021; Lin et al., 2020).

The majority of the time, ARG is confined in plasmids or integrons and is horizontally transmitted into bacteria as intracellular ARG (iARG) or intracellular DNA (iDNA) or as extracellular ARG (eARG) or extracellular DNA (eDNA) that exists in the external environment (Lin et al., 2020). As we already know one of the ways that AR is transmitted naturally is by horizontal gene transfer (HGT) through its three different mechanisms—conjugation, transformation, and transduction—are used to induce bacteria to acquire AR (Yang et al., 2013a). Conjugation is accomplished by creating a channel between bacteria using fimbriae; transformation is a process in which capable recipient bacteria consume naked ARG and develop resistance; and transduction is the transmission of ARG from donor bacteria to recipient bacteria via phages (Frost et al., 2005). The hazard of the spread of AR is increased by the accumulation of ARB in the natural environment and their potential to move ecologically with eARG. The "Engineering Fronts 2019" study, which was published in 2019 by the Center for Strategic Studies of the Chinese Academy of Engineering, placed special emphasis on the value of research into the features of AR pollution and the law of migration and transmission (Lin et al., 2020). This shows that both the general public and the scholarly community have given the migration and spread of AR in the natural world a great deal of attention. A variety of environmental or biological mediums can disseminate AR contamination. As a result, the spread of AR contamination will depend heavily on environmental media migration in addition to horizontal transfer (Lin et al., 2020). While at the same period, HGT is anticipated to continue to occur when AR migrates through environmental media. This implies that the spread of AR contamination may be accelerated by the interaction of several migratory strategies. Understanding the various AR pollution migration pathways is essential for preventing the spread of AR in the ecology.

Three different characteristics help to illustrate the delicate parameters that affect how AR is distributed and spread: horizontal gene transfer, the distribution of prospective host bacteria in the environment, and the mobility of ARG and ARB in environmental media are all variables (Ojha et al., 2021; Lin et al., 2020). In this study we have already discussed how AR is spread and distributed through HGT and its different mechanisms.

Distribution of prospective host bacteria with ARG in the environment

By eliminating the components required for bacteria or fungus to carry out their regular life processes, antibiotics can have antibacterial or bactericidal effects (Lin et al., 2020). Antibiotic overuse, however, has resulted in the development of antibiotic-resistant diseases and also super bacteria (Naimi et al., 2001; Walsh et al., 2011). Already, ARB pose a health hazard. Studies have demonstrated the ability of competent bacteria, such as Acinetobacter, Haemophilus, Neisseria, Pseudomonas, Staphylococcus, and Streptococcus, which are numerous human pathogenic bacteria with inherent transformation capacities, can collect free DNA containing ARG in the environment (Traglia et al., 2014; Johnston et al., 2014). As a result, research on ARB is becoming increasingly popular, and the relationship between ARB and ARG has steadily taken center stage. It is a good method to follow ARG host bacteria in order to analyze the interaction amongst the ARG and bacterial populations because of the non-random co-occurrence of ARG and bacterial taxa (Chen et al., 2017). Table 1 (Lin et al., 2020) exhibits a list of few partial resistant genes and their host bacteria.

In a research on the isolation of ARG-bearing strains in freshwater habitats, Nnadozie and Odume (2019) provided a summary and showed that there are many ARB and ARG in freshwater habitats. The study of urban water mains and wastewater treatment processes has also revealed the widespread occurrence of ARB and ARG. The tetracycline and sulfonamide ARG predominated among the 16 types of ARG that Yao et al. (2019) found in the wastewater treatment plant's water body. They also discovered the existence of the mobile genetic element intI1, which is intimately linked to the movement and transformation of ARG. The wastewater

treatment facility plays a significant role in the urban water drainage system. It is a source of contaminated surface water in addition to being a site where different types of sewage are collected. Consequently, through urban runoff water, ARG or ARB found in wastewater, soil, sludge, etc., can travel further into natural aquatic environment and constantly accumulate there (Almakki et al. 2019). In addition, some aquatic settings are specifically employed for breeding, such as offshore breeding (Suzuki et al. 2019). The ocean is one of the major sources of ARG migration and proliferation in the natural habitat due to the abundance of ARB and ARG enrichment.

Table 1: Genes for partial resistance and their host bacterium

ARG	Host Bacteria	Origin	Reference
bla$_{NDM-1}$	*Klebsiella pneumoniae*	Hospital	Yong et al. (2009)
sul1, sul2	*Bacillus methylotrophicus, Acinetobacter sp, Klebsiella pneumonia, Enterobacter hormaechei, Serratia marcescens, Staphylococcus saprophyticus*	Pharmaceutical wastewater plant	(Obayiuwana et al. 2018)
bla$_{CTX-M}$, bla$_{TEM}$, qepA	*E. coli*	Hospital wastewater	(Lien et al. 2017)
bla$_{SHV}$, bla$_{TEM}$, bla$_{OXA}$, bla$_{CTX-M}$	*E. coli, M. morganii*	Retail food	(Ye et al. 2017)
VanC	*E. casseliflavus*	River	(Nishiyama et al. 2017)
mecA	*Staphylococcus aureus*	Surface waters	(Hatcher et al. 2016)

Continuity of Table 1

vanA	*Enterococcus faecium*	Rural aqueous environment	(Morris et al. 2012)
bla$_{KPC-2}$	*E. coli, K. pneumoniae, Enterobacter cloacae*	Spanish river ecosystem	(Piedra-Carrasco et al. 2017)
vanA, vanB, vanC1	*Enterococci faecium*	Hospital sewage	(Novais et al. 2005)

Transmission of Antibiotic Resistant in the Environment

The contact of ARG with the ambient surface, as opposed to the horizontal transfer of ARG in the ecosystem, is fundamental to the movement of ARG. ARG can also be found in extracellular polymers (EPS; for instance, proteins, lipopolysaccharides, and extracellular polysaccharides), cell detritus, or on the surface of minerals in addition to the genomic or plasmid DNA seen in ARB (Ojha et al., 2021; Dodd 2012). Environmental media that contain soluble EPS can circulate freely across them and are not readily trapped by solid deposits (He et al. 2019). The probability of resistance spread may rise if soluble EPS contains eARG. Additionally, there are far more extracellular free DNA molecules containing ARG than iDNA in environmental media (Mao et al. 2014). Furthermore, only a few number of research have demonstrated that pH has a major impact on natural transformation (Ojha et al., 2021; Traglia et al. 2016), which indirectly influences how eARG interacts with environmental media. More research is essential to determine the impact of other environmental conditions on eARG adsorption and retention.

exists between environmental media can be moved unrestricted and is not easily captured by solid deposits (He et al. 2019b). If soluble EPS contains eARG, the risk of resistance spread may increase. And the number of extracellular free DNA carrying ARG found in environmental

media is much greater than iDNA (Mao et al. 2014). In addition, only a few studies have shown that pH has a significant effect on natural transformation (Traglia et al. 2016), which indirectly affects the interaction of eARG with environmental media. Further studies on the effects of other environmental factors on the adsorption and retention of eARG are needed (Ojha et al., 2021; Lin et al., 2020). Additionally, biofilms can be created by microbial populations that are adhered to the surface of certain soil or sediment particles. Studies have demonstrated that biofilms can prevent resident organisms from being stressed by the environment and have a substantial impact on how bacteria propagate in water bodies (Parsek and Singh 2003), soil, and aquifers (Ojha et al., 2021; Klayman et al. 2009). Biofilms are common in the aquatic environment and are crucial to many ecological and biological processes (Guo et al. 2017). According to Wang et al. (2015) and Zeng et al. (2015), biofilms have a great potential to absorb a variety of contaminants, including metals, leftover antibiotics, and resistance genes. They may also act as ARB binding sites, facilitating AR's spread and acquisition.

The Antibiotic Resistome

The total number of antibiotic resistance genes found in pathogens, bacteria that manufacture antibiotics, and good environmental bacteria is known as the resistome. In addition, the resistome contains precursor genes which codes for metabolic or housekeeping functions, which we have named protoresistance elements because resistance arises from these genes (Institute of Medicine et al., 2011; Morar et al., 2009). Last but not least, the intricate interaction of genes and their byproducts resulting from exposure to hazardous toxins might result in antibiotic resistance even at the organism level (Breidenstein et al., 2008; Institute of Medicine et al., 2011; Fajardo et al., 2008; Tamae et al., 2008). As a result, the inherent systems biology of organisms that leads to an organism evading the activity of antibiotics is also a part of the resistome. A conservative perspective of antibiotic resistance holds that processes that produce resistance inside one organism, regardless of whether pathogen or not, have the ability to develop into clinically significant microorganisms. This is due to HGT between microorganisms of various species as well as genera (Institute of Medicine et al., 2011; Wright, 2010). The development of new antibiotics and the maintenance of the ones currently in use are dependent on our capacity to

understand the resistome as a whole, rather than just as a collection of mechanisms that have already evolved in bacteria. Several common pathways can lead to antibiotic resistance. Among them are: (i) Chemically or genetically modifying the macromolecular therapeutic target to produce insensitive mutants. (ii) Immunity proteins are produced to protect the target. (iii) Direct chemical modification of the antibiotic, generally by the activities of enzyme catalysts. (iv) Alternate solutions include altering the way that substances are transported into cells. (v) Drug leaving the cell through increased efflux (Wright, 2010). This analysis of resistance mechanisms should also take into account physiology-based or innate genotype-based intrinsic insensitivity to antibiotics. These can include the presence of cell structures such as Gram-negative bacteria's relatively impermeable outer membrane, physiologically dormant states such as biofilms or spores, and the inherent redundant interconnection of proteins and genes that contribute to antibiotic resistance, which are frequently species-specific (Institute of Medicine et al., 2011). Because a drug-sensitive target is often present, these processes may be seen as antibiotic insensitivity rather than resistance. However, from the perspective of both consumers and discoverers of antibiotics, such drugs are ineffective, hence we include such a process within resistome (Institute of Medicine et al., 2011). As a result, the resistome is a complex collection of genes and biological processes that operate either explicitly or implicitly to reduce the effect of antibiotics (Institute of Medicine et al., 2011; Wright, 2010). The fact that resistant strains frequently carry many mechanisms, leading to cumulative resistance (Figure 8), further confuses the problem and makes it difficult to comprehend how each mechanism contributes to the overall phenotype. This, in turn, makes clinical management more difficult.
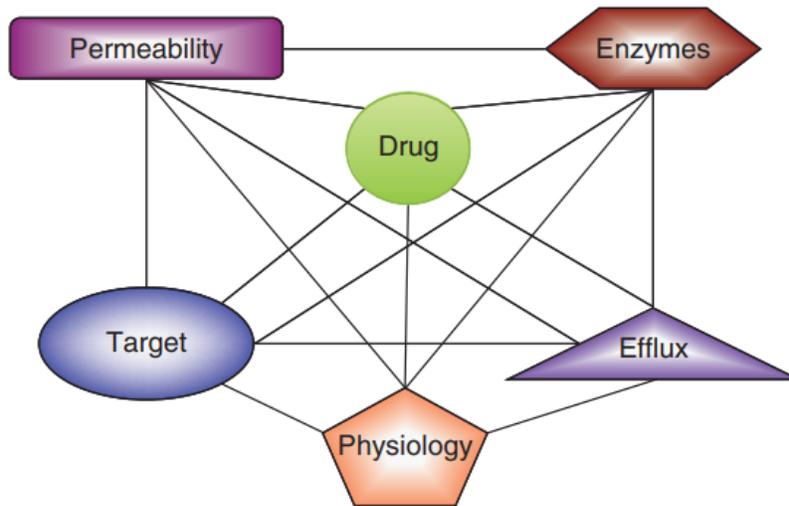
Figure 8: Cumulative Resistance. Collective Antibiotic resistance in organisms frequently results from many pathways, including alternative transport, the physiological condition of the cell, such as biofilm formation and development, and the availability of modifying enzymes. The graphic depicts potential routes for antibiotic resistance, and the interconnecting lines represent known combinations of resistant microorganisms. Not all ingredients are present in all resistance species, but fusion of two or more are frequently encountered, resulting in a chemical-genetic profile with a high degree of connectivity that is difficult to overcome and confers resistance (Wright, 2007; Wright, 2010).

The Clinical Antibiotic Resistome

The resistome in clinical practice for good reason, most doctors and medical microbiologists limit their antibiotic resistance research to therapeutically significant organisms. For therapeutic usage, treatment, and research, it is essential to comprehend the processes, transmission, and epidemiology of pathogenic bacterial resistance (Institute of Medicine et al., 2011). As previously noted, unlike adaptable infections of natural derivation, the bacteria that are commonly linked with infection are or used to be antibiotic-resistant, and there is substantial evidence that disease resistance development is frequently the consequence of natural selection

inside the clinic. For example, an analysis of the Murray collection of enterobacteria (433 stains) collected between 1917 and 1952 found that, whereas pre-antibiotic usage bacterial isolates included conjugative plasmids competent of horizontal gene transfer, neither one of these possessed resistance genes (Institute of Medicine et al., 2011; Hughes & Datta, 1983). Greater incidence of resistance, on the other hand, is normal and predicted after the introduction of antibiotics in a single patient, a clinical care system, or across populations (Grayson et al., 1991; Institute of Medicine et al., 2011; Hawkey & Jones, 2009; Livermore, 2009). RNA polymerase gene rpoB point mutations that result in rifampin resistance, DNA gyrA and topoisomerase parC mutations that result in fluoroquinolone resistance, as well as rpsL and streptomycin resistance, are only a few examples of single mutants that can induce clinical resistance (Institute of Medicine et al., 2011). These alterations typically reduce a drug's ability to successfully connect to a target. Resistance can be conferred by mutations that upregulate genes, which is frequent with efflux mechanisms. On the other side, downregulating transport proteins like porins, which block the passage of antibiotics into the cell, can induce resistance. The acquisition of genes and their steady integration onto the bacterial genome is another form of mutation that leads to resistance. An illustration of this mechanism in MRSA is the acquisition of the SCCmec cassette or staphylococcal cassette chromosome mec (Institute of Medicine et al., 2011; de Lencastre et al., 2007). Such strains are clonally disseminated, resulting in resistant populations that may have geographical boundaries, such as to particular hospital facilities or wards. Due to selection pressures brought on by drug usage, the founder strains may prevail in such situations. Bacterial genome sequencing, for instance, can be used to identify lineages and trace the natural history of clinical resistome associated with a specific epidemic. While genotype analysis can be challenging in this situation, resistance elements can move across strains via HGT, leading to rapid adaptation and radial gene dispersion into several strains, of which each has the capacity to be a founder. HGT can occur by conjugation, transduction, or transformation. Plasmid-mediated HGT has the capacity to disseminate resistance genes throughout microbiomes (Institute of Medicine et al., 2011). Plasmids have accumulated more resistance genes since the 1940s with the introduction of antibiotics; these genes are typically on transposable elements that allow gene transfer onto the chromosome (Institute of Medicine et al., 2011; Barlow, 2009). Screening for resistance to one class of antibiotics may inadvertently result in co-selection for genes giving resistance to structurally different antibiotics because resistance genes are grouped onto mobile

genetic elements such as plasmids and otherwise transposons. The clinical resistome is difficult to map and model due to its mobility and co-selection. Although the origin of the resistance genes present on these genetic components is unknown, the expanding knowledge of the size of the environmental resistome, as stated above, strongly implies a connection. The assumption that the environment is the primary source of a significant component of the clinical resistome is supported by the abundance of resistance genes as well as organisms in the ecosystem (Institute of Medicine et al., 2011). One illustration of a possible link is the similarities of the vancomycin resistance gene cassette in pathogenic and environmental organisms. Another is the relationship between the CTX-M prolonged spectrum b-lactamases, which are widely used in clinics worldwide, and the reservoir in the environmental bacteria Kluyvera ascorbata (Institute of Medicine et al., 2011; Humeniuk et al., 2002).
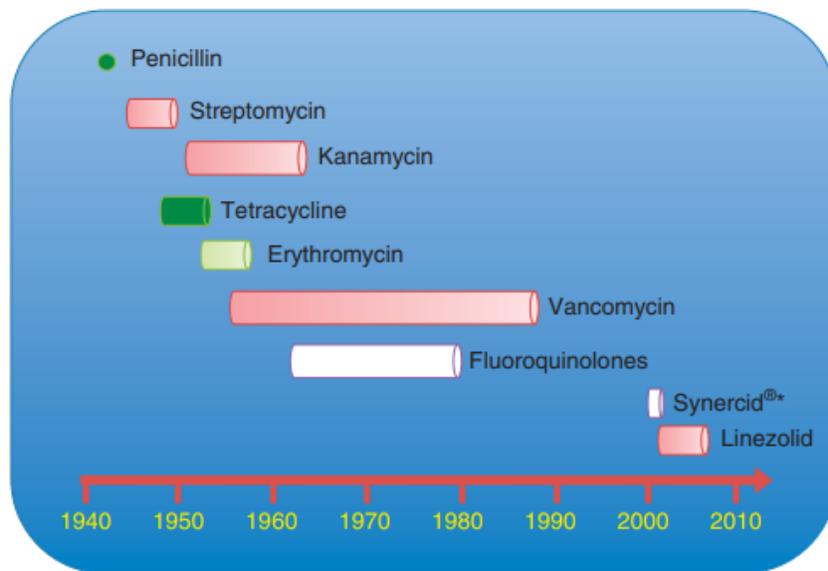

Figure 9: The development of antibiotic resistance. an overview of specific instances of the introduction of antibiotics into clinical settings and the development of pathogen resistance. The commencement of the bar indicates the start of clinical usage, and the bar is terminated when pathogen resistance becomes a substantial clinical problem. Note that resistant microorganisms for penicillin (here shown by a circle) were discovered long before the antibiotic was put to widespread clinical use (Institute of Medicine et al., 2011; Wright, 2010; D'Costa et al., 2007;

D'Costa et al., 2006; Wright, 2007). *Dalfopristin and quinupristin, an antibiotic combination, are sold under the brand name (Wright, 2007).

The Intrinsic Antibiotic Resistome

Antibiotic resistance and sensitivity vary widely among bacteria. A species' (and even a strain's) inherent resistance to medications is determined by the presence or lack of resistance-related genes. The intrinsic resistome is made up of this set of genes (Breidenstein et al., 2008; Institute of Medicine et al., 2011; Fajardo et al., 2008; Tamae et al., 2008). These genes can sometimes be easily identified as being a part of well-known resistance gene families. Because this species has the Lsa efflux protein, Enterococcus faecalis is, for instance, consistently resistant to the antibiotics lincosamide and streptogramin (Singh et al., 2002). As was mentioned earlier, opportunistic organisms like Psudomonas aeruginosa encode several resistance genes, especially efflux proteins that offer widespread drug resistance. Other comprehensive investigations have shown a cluster of genes that support intrinsic resistance in addition to these well-known components. Studies on the interactions between chemicals and synthetics have proven very instructive. In these experiments, a library of mutants are screened for sensitivity to or resistance to antibiotics at doses that are not lethal. Candidates for the intrinsic resistome are mutations that increase sensitivity to the antibiotic. These may serve as targets for novel medications that enhance the effects of antibiotics. One warning is that these research' design prevents important genes from being sampled, which could lead to an underestimation of the intrinsic resistome's size. (Wright, 2010). A library of P. aeruginosa PA14 transposon mutants was used to screen the fluroquinolone antibiotic ciprofloxacin, and 35 mutants with increased sensitivity and 79 mutants exhibiting lower sensitivity were found (Institute of Medicine et al., 2011; Breidenstein et al., 2008). The genes linked to intrinsic resistance included expected efflux systems as well as less obvious ones, like the ClpX and ClpP proteases and genes associated with DNA replication, repair, as well as cleavage. The recent finding that acyldepsipeptide antibiotics target fluoroquinolones opens the possibility of synergistic combinations with these drugs (Institute of Medicine et al., 2011; Brötz-Oesterhelt et al., 2005). In a related study, Fajardo et al. tested a panel of six antibiotics representative of various antibiotic classes against two libraries of P.

aeruginosa transposon mutants. Their research found that some mutants elevated sensitivity to several antibiotics, indicating a severe loss of discrimination through the genetic networks which guard the cell against harmful molecules (Institute of Medicine et al., 2011). (2008). Acinetobacter baylyi transposon mutant library underwent a comparable screen, it was reported (Gomez & Neyfakh, 2006). The effects of 12 antibiotics at sub-inhibitory concentrations were examined, and 11 genes with chemically synthesized fatal phenotypes were found. Several genes associated with the efflux system and cell wall metabolism, in contrast to the Pseudomonas screens previously mentioned, were unrelated to recognized antibiotic targets as well as resistance elements (Institute of Medicine et al., 2011). After systematically analyzing the sensitivity of four thousand single-gene deletion strains of Escherichia coli to seven antibiotics, 140 distinct synthetic chemical lethal interactions were finally identified (Institute of Medicine et al., 2011; Tamae et al., 2008). This work has subsequently been extended to include 22 antibiotics, supporting the intrinsic resistome genetic network's complexity and producing a unique sensitivity profile that can predict an antibiotic class (Liu et al., 2010; Institute of Medicine et al., 2011). During the course of the drug development process, the categorization of antibiotics may be done using this cellular bar code.

Adept Outlook into Antibiotic Resistome

Past studies on antibiotic resistance have mostly concentrated on the epidemiology and development of resistance in clinical infections. Because of advances in our understanding of the molecular mechanisms underlying resistance, as well as our understanding of the 3D structures of such elements and the prevalence of several related resistance genes in non-pathogenic microbes, it is becoming increasingly clear why resistance is so common and appears so quickly after the application of antibiotics in clinical settings. Additionally, the resistome idea explains why resistance-proof medications are a myth by demonstrating the extraordinary depth of the gene pool to source resistance and the simplicity of HGT in bacterial populations (Institute of Medicine et al., 2011). Complete knowledge of the specific processes of HGT in the environment is still missing, and there aren't enough instances of recent HGT from ambient organisms to diseases to be sure of it. Despite the resistome's presence throughout this period, antibiotics have

proven to be miraculous cures and extremely profitable for the pharmaceutical industry over the previous six decades.There is much cause for optimism because comprehending the resistome opens up new doors in the realm of medication research. First, conventional methods of discovering new antibiotics from both natural and synthetic sources have been successful, and they should be continued. Early in the discovery phase, screening environmental organisms for drug resistance to potential candidates might aid in identifying protoresistance and genuine resistance factors that may later surface in clinical settings. This can be utilized to decide between competing compounds during the preclinical discovery phase or for lead optimization. Additionally, it could be able to spot potential resistance components in infections, opening up the possibility of using diagnostic testing before or after clinical trials. Second, considering medication combinations in antibacterial therapy is a logical consequence of the resistome idea. Antibiotic combinations are frequently used in the treatment of infectious diseases, however, there are not many developed combinations. The discovery of bacteria's intrinsic resistome has provided a number of potential targets for inhibitors of non-essential gene derivatives that might be used in combination with known antibiotics. This strategy has several difficulties, such as matching the pharmacological characteristics of the bioactive components in any combination medication formulation, regulatory obstacles, and clinical trial design issues. However, as the incidence of multidrug-resistant bacterial diseases develops in the healthcare industry and in the community, exploiting the resistome by means of medicine combinations will become more tempting. Wright (2010); Institute of Medicine et al., 2011).

Conclusion

More resistant strains of AR are produced as a result of AR's horizontal transfer and migration in the environment. In contrast to horizontal transfer, ARB and ARG move and proliferate in the environment over time, continually enriching it and causing AR to spread more extensively. There might be horizontal transfer of ARG during this phase. The majority of recent research has focused on the horizontal transfer of AR at the cell scale, and there has been relatively limited research of AR migration and dispersion at the environmental scale. iARG and eARG are also infrequently differentiated in current studies on the spread of AR. In contrast to eARG, which is DNA free of extracellular cells and has a substantially greater diffusion impact than iARG, iARG can migrate and diffuse by analyzing ARB in the environment. Consequently, independent studies should be conducted on the enrichment and dispersion of eARG and iARG in the environment. The nature of free DNA and ARB in the environment makes it potentially challenging to conduct such research. This has made it challenging to fully comprehend how AR pollution spreads across the ecosystem and presents novel challenges for researchers looking into AR's effects on the environment. Additionally, the notion of the resistome, which demonstrates the extraordinary depth of the gene pool to source resistance and the simplicity of HGT in bacterial populations, explains why resistance-proof drugs are a popular misconception. An in-depth understanding of the specific processes behind HGT in the environment is still inadequate, and there is a severe paucity of examples of recent HGT from ambient organisms to diseases that are unquestionably true. Future research should examine how much antibiotics' modulation of HGT contributes to the spread of resistance. In order to reach definitive insights, we underline the necessity for well planned quantitative research and consistent definitions of HGT efficiency for each modality of transfer. In order to properly quantify HGT, especially in more complicated contexts with extra populations or spatial limits, it is necessary to understand how various selection regimes affect many populations participating in the process. Designing efficient treatment plans aimed at reducing the spread of resistance requires an understanding of how antibiotics alter HGT efficiency factors. While we queue for the discovery of novel antibiotics, some of which may even target HGT, such research can help us use already available antibiotics more effectively.

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