Issue 17, Volume 1 December 2024

A Literature Review on the Evolution of Antibiotic Resistance and its Impact

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ACKNOWLEDGEMENTS

This paper was written for BIO 141: Evolution.

The Evolution of Antibiotic Resistance

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BIO 141: Evolution

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April 27, 2024

Abstract

The widespread use of antibiotics has revolutionized modern medicine, helping to fight against countless bacterial infections, significantly reducing mortality rates, and preventing the further spread of bacterial diseases. However, the overuse and misuse of antibiotics have come with a significant downside: they have allowed bacteria to develop antibiotic resistance, posing a major challenge to public health and the effective treatment of infectious diseases. Antibiotic resistance occurs when bacteria evolve and adapt to withstand the effects of the antibiotics designed to kill them. This phenomenon poses a great threat to global health, complicating the treatment of patients and increasing the risk of severe illnesses, death, and disease spread. Bacteria have evolved to develop antibiotic resistance through various mechanisms, including genetic mutations and the process of horizontal gene transfer. Additionally, other non-genetic factors such as ecological contexts and interspecies interactions, play a crucial role in the evolution and spread of antibiotic-resistant bacteria. The effects of antibiotic resistance worldwide result in prolonged hospital stays, increased healthcare costs, and higher mortality rates. In this review, I explore the phenomenon of antibiotic resistance in bacteria, how it has evolved, and its impact on society, while emphasizing the importance of developing new strategies to combat this growing threat.

Keywords: antibiotic resistance, horizontal gene transfer, conjugation, mutation

The Evolution of Antibiotic Resistance

Antibiotics are revolutionary in modern medicine, enhancing treatments against a variety of microorganisms. The first antibiotic was Salvarsan, developed by Paul Ehrlich to treat *Treponema pallidum* which causes syphilis, making the first class of synthetic arsenic-based pro-drugs (Hutchings et al., 2019). Gerhard Domagk, another scientist, wanted to continue Ehrlich's work and developed penicillin, making a breakthrough in the scientific community (Hutchings et al., 2019). This discovery inspired many scientists to work with microbes and initiated a Golden Age of antibiotic discovery, making new antibiotics yearly (Hutchings et al., 2019). However, the vast development of antibiotics and their usage in many areas now pose a great challenge for the treatment of disease due to the rise of antibiotic resistance.

Beyond drug development, antibiotics are continuously used in nonmedical settings. In agriculture, they are used as growth promoters for animals, added to their food and water to increase their size; in veterinary medicine, they are used to treat animals; and in aquaculture, to farm fish. Domestically, antibiotics are found in daily products such as shampoo, soap, cleaning products, and detergents (Meek et al., 2015).

The development of antibiotic resistance by bacteria is a prime example of evolution. Antibiotic resistance occurs when the bacteria usually inhibited by antibiotics can resist the antibiotic's effects and evade these conditions. With the rise in antibiotic usage worldwide, comes the rapid rise of antibiotic-resistant bacteria, increasing their capacity to defeat the drugs designed against them (Davies & Davies, 2010). In 2013, it was found that each year, 2 million people in the United States are infected with antibiotic-resistant bacteria, and at least 23,000 die

from these infections (Christaki et al., 2019). Worldwide, 700,000 people die annually, and this number is estimated to rise to 10 million deaths per year by 2050 (Mancuso et al., 2021).

Additional impacts are seen in animals where antibiotic-resistant bacteria were found in poultry and have been linked to human infections (Meek et al., 2015). Other social impacts include healthcare where individuals have extended work leave due to prolonged medical care as a result of infections and increasing hospital stays (Muteeb et al., 2023). Further implications of antibiotic resistance include economic losses where the development of resistant bacteria in animal husbandry can disrupt food production and increase its cost (Muteeb et al., 2020). This calls for increased surveillance of antibiotic consumption, usage, and increased research in antibiotic development and treatments against bacterial diseases.

Literature Review: The Evolution of Antibiotic-Resistant Bacteria

Mechanisms that lead to Antibiotic Resistance

Throughout generations, bacteria have evolved to become more resistant to antibiotics through different mechanisms, mutations, and gene transfer processes such as horizontal gene transfer (MacLean et al., 2019). Bacterial resistance can be of three types: intrinsic, adaptive, and acquired. Intrinsic resistance can be due to the inherent properties of the bacteria itself (Christaki et al., 2019). An example of intrinsic resistance is glycopeptide resistance by gram-negative bacteria. Glycopeptide resistance is resistance against glycopeptide antibiotics that arise due to cell wall peptidoglycan precursor modifications. The terminal D-Ala-D-Ala amino acid sequence is altered by bacteria such as *Enterococci*, reducing the binding of the antibiotic, and evading the effects of glycopeptide antibiotics (Christaki et al., 2019). Bacterial cells can keep the intracellular concentration of the antibiotic low by decreasing or mutating the proteins in their

outer membrane, porins, to prevent the entry of antibiotics into the cell (Huemer et al., 2020). This resistance is a naturally occurring process that existed long before the widespread use of antibiotics (Mancuso et al., 2021). The second type of resistance is adaptive resistance. Adaptive resistance is temporary resistance due to environmental signals. This type of resistance is induced by environmental signaling changes such as stress, pH, and nutrient conditions that alter gene expression in the bacteria. Once the environmental signals are removed, the bacteria return to their normal state (Christaki et al., 2019). Lastly, the most important method of bacterial antibiotic resistance is acquired resistance. Acquired resistance is resistance acquired due to the incorporation of foreign genes or through mutations in the bacterium's genes (Christaki et al., 2019).

There are various mechanisms bacteria can use to inhibit the effect of antibiotic resistance. Such mechanisms include target modification by enzymes, target replacement, target protection, reduction of cell permeability, and efflux pumps (Muteeb et al., 2019). Bacteria have ß-lactamase enzymes that destroy the amide bond of the ß-lactam ring in the antibiotic, making the bacteria resistant, and rendering the drug ineffective (Christaki et al., 2019). Genes encoding ß-lactamase enzymes were identified in over 1000 bacteria and the genes coding for these enzymes were found in mobile genetic elements that can spread across bacterial populations (Christaki et al., 2019). Antibiotics, such as aminoglycosides, can inhibit ribosome assembly and prevent protein synthesis in bacteria; however, this effect can be inhibited through antibiotic modification (Mancuso et al., 2021). Bacteria can modify aminoglycosides by enzymes such as aminoglycoside modifying enzymes (AME) that can phosphorylate, acetylate, or adenylate the drug, modifying it, and making the bacteria resistant to this antibiotic (Christaki et al., 2019).

Bacteria can further reduce the concentration of intracellular antibiotics by increasing the activity of efflux pumps (Huemer et al., 2021). Efflux pumps are energy-dependent proteins in the cytoplasmic membrane of bacteria that pump toxic molecules, such as antibiotics, outside of the cell (Christaki et al., 2019). An example is the efflux pump that pumps tetracycline (an antibiotic) out of the cell in *E. coli* which is encoded by a plasmid, a circular piece of DNA that contains unessential, but beneficial genes for the bacterial host cell (Christaki et al., 2019). Other efflux pumps such as multidrug efflux pumps are chromosomally encoded, increasing the resistance of bacteria to antibiotics, but only a few provide relevant clinical resistance (Christaki et al., 2019). Other pumps such as substrate-specific pumps are in mobile genetic elements that can be transferred across bacterial populations, increasing the spread of antibiotic resistance (Christaki et al., 2019). These mechanisms of resistance allow antibiotic-resistant bacteria to have a selective advantage over other bacteria, being favored by natural selection to survive and reproduce (Meek et al., 2015). By studying these mechanisms, drugs could be developed to interrupt or mutate these proteins to prevent their function against antibiotics.

Mutations

Mutations are one of the primary ways by which bacteria can develop antibiotic resistance. Mutations are random changes in DNA that could lead to changes in the nucleotide sequence and increase genetic diversity among the bacterial community. These changes in DNA can occur due to evolution and transfer from one generation to the next (Muteeb et al., 2021). In many instances, the newly taken genes can encode proteins that aid in antibiotic resistance. Researchers found that mutations in any of the following three types of genes could be factors in the evolution of antibiotic-resistant bacteria. These types of genes include those that encode the

target of the antibiotic, their transporters, or regulators that repress the expression of their transporters (Martinez, 2014). Point mutations can increase the diversity of phenotypic variants in bacteria, giving some bacteria a selective advantage over others and favoring their selection (Baquero & Blazquez, 1997).

The presence of antibiotics can influence mutations that affect the expression and function of porins, proteins in the outer membrane of the bacteria. These mutations can lead to the complete loss of porins, size modification, or reduced expression (Christaki et al., 2019). It is important to note that reduced expression of porins leads to only low-level antibiotic resistance but it can be enhanced by the presence of other resistant mechanisms such as efflux pumps and antibiotic degrading enzymes (Christaki et al., 2019). Mutations in genes encoding regulatory systems such as metabolism and homeostasis can lead to daptomycin (another antibiotic) resistance. Similarly, *Staphylococcus aureus,* a common bacterium found on human skin, can develop resistance to vancomycin, another type of antibiotic, due to changes in those homeostasis regulatory genes (Christaki et al., 2019). Such changes can reduce cell wall turnover, autolytic activity, and increase cell wall synthesis, preventing vancomycin from entering the cell and reaching its target (Christaki et al., 2019). Mutations can also affect the target sites of antibiotics. These mutations can alter the structure of the antibiotic binding site, reducing the affinity of the antibiotic to its target, rendering it less effective, and increasing the resistance of the bacteria toward it (Muteeb et al., 2021). On the other hand, mutations can code for proteins that enhance resistance. One example is how mutations in the ribE gene can code for a lumazine synthase enzyme that can play a role in nitrofurantoin (another type of antibiotic) resistance (Christaki et al., 2019). The bacteria that survive the effects of the antibiotic will have a selective advantage

over the rest of the bacterial population, successfully reproducing and passing on their traits through vertical transfer, the passage of DNA from mother to daughter cells.

Horizontal Gene Transfer

Bacteria can acquire foreign antibiotic-resistant genes through the process of horizontal gene transfer (MacLean & Millan, 2019). Horizontal gene transfer is the transfer of genes, different from mother to daughter (vertical transfer), but from one unrelated bacterium to another through three mechanisms, conjugation, transduction, and transformation. Each of these mechanisms is summarized in Figure 1. Horizontal gene transfer methods allow the movement of antibiotic resistance genes across bacterial populations and lead to bacterial evolution (Burmeister, 2015).

Conjugation. Conjugation is one of the most common methods of transfer of antibiotic-resistant genes from one bacterium to another (Bello-López et al., 2019). Conjugation is the direct transfer of genetic material from one bacterium to another through direct contact and the extension of a pilus as a source of transport (McInnes et al., 2020). This allows the transport of mobile genetic elements such as plasmids and ICEs (integrative and conjugative elements), allowing the transport of non-self-transmissible DNA (McInnes et al., 2020). Plasmids are circular pieces of DNA that contain unessential but beneficial genes for the host (Bello-López et al., 2020). Unlike transduction and transformation, conjugation requires physical contact between the two bacterial cells (Figure 1). The donor cell makes a pilus using a protein encoded in the plasmid (prop-pilin) and it forms a bridge that allows the recipient to get a copy of the plasmid (Bello López et al., 2020). Once the recipient receives the plasmid, they acquire all the genes the plasmid contains (Bello-López et al., 2020). Many plasmids contain genes that provide antibiotic

resistance; therefore, the transfer of such genes can lead to the evolution of antibiotic-resistant bacteria. Using this knowledge, scientists could explore methods that can block conjugation by mutating the genes that form a pilus between the donor and recipient cell, limiting the spread of resistant genes across bacteria.

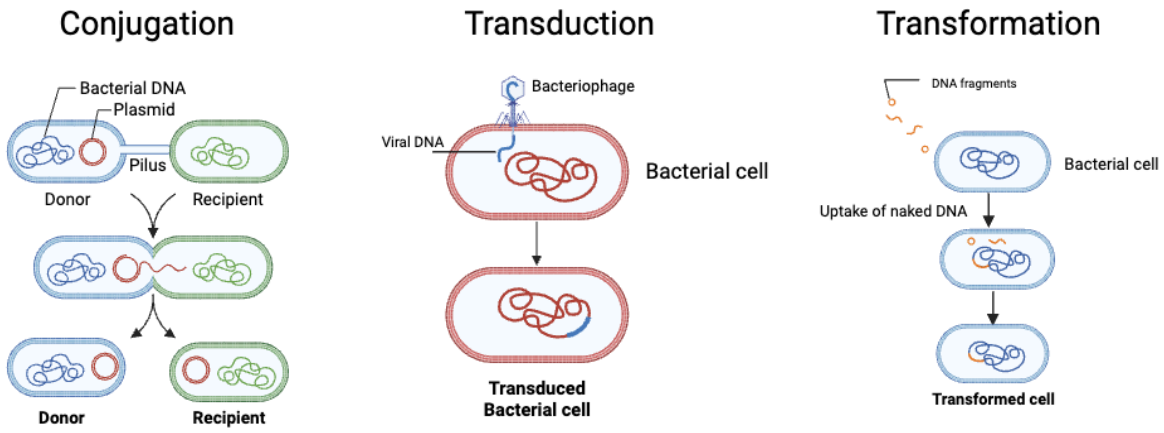
Transduction. Transduction is the second mechanism of horizontal gene transfer (Figure 1). It is the transfer of random DNA through a bacteriophage (viruses that infect bacteria) and transmitting it to another bacteria (Burmeister, 2015). There are three main mechanisms of transduction which include generalized, specialized, and lateral transduction which allow the transfer of any part of the bacterial genome from one to another (McInnes et al., 2020). Generalized transduction is when a bacteriophage packages some of the host DNA in its capsid while the cell is in the lytic cycle (McInnes et al., 2020). Specialized transduction is when regions of DNA adjacent to the integration site of the lysogenic phage are packaged into the bacteriophage capsid (McInnes et al., 2020). Lastly, lateral transduction is when prophages (parts of the viral genome integrated into the host genome) initiate DNA replication to assemble new bacteriophages that lead to cell lysis and spread to other cells (McInnes et al., 2020). Once bacteriophages spread, they can inject the DNA into another host cell, integrating into its genome (Bello-López et al., 2020). The process of transduction allows the transfer of antibiotic-resistant genes among bacteria. An example of this was seen in the human gut where many phages were found to carry antibiotic-resistant genes after antibiotic treatment, promoting genetic diversity, and increasing the spread of antibiotic resistance across *E. coli* (McInnes et al., 2020).

Transformation. Transformation is the process of acquiring DNA from the environment and inserting it into the bacterial genome (Sun et al., 2019). When bacterial cells die, their DNA

can be released into the environment and can be taken in by other cells if they are competent (Figure 1). Bacteria can be competent for transformation by having the necessary membrane channels and being in an appropriate state which could be due to a lack of nutrients or an elevated cell density (Bello-López et al., 2020). This process of random uptake of DNA from the environment poses a great challenge for researchers and scientists around the world since it allows for consistent change in bacterial genomes. As more bacteria become competent to take DNA from the environment, the more diverse the bacterial population becomes, making it more challenging to develop drugs and treatments against bacterial infections. Therefore, the process of horizontal gene transfer is important for the spread of antibiotic-resistant genes across bacteria, and it allows bacteria with those genes to be selected for by natural selection, passing on those genes to their offspring.

Figure 1.

Summary of the Three Mechanisms of Horizontal Gene Transfer: Conjugation, Transduction, and Transformation



Note. Conjugation is the transfer of plasmid DNA from the donor to the recipient through a pilus and direct cell-to-cell contact. Transduction is the transfer of viral DNA into a bacterial cell

through a bacteriophage and its integration into the bacterial genome. Transformation is the uptake of naked DNA fragments from the environment into a bacterial cell and integrating it into the bacterial genome.

Ecological Context and How It Affects Antibiotic Resistance

Besides gene transfer across bacterial populations, bacterial responses to antibiotics can be affected by interacting microbes (Bottery et al., 2020). In biofilms, collections of microorganisms attached to a cell surface, bacteria can have an increased level of resistance by altering the expression of pre-existing antibiotic-resistant genes (Bottery et al., 2020). Exotoxins, soluble proteins secreted by bacteria that enter the host cell and alter its physiology to disrupt cellular functions, can help other species of bacteria transition into a tolerant state against the antibiotic (Barbieri, 2009; Bottery et al., 2020). An example of this is *P. aeruginosa,* a gram-negative bacterium known for causing infections, which secretes 4-hydroxy-2-heptylquinoline-N-oxide, a compound that inhibits the electron transport chain (ETC) and slows down the growth of *S. aureus*, another bacterium, allowing it to be in a highly tolerant state (Bottery et al., 2020). This highlights microbial interactions that can increase other bacterial tolerance to antibiotics, giving room for the survival and selection of pathogenic bacteria, despite the use of antibiotics. This shows that improved knowledge of microbial ecology and interactions can help scientists further identify ways to disrupt or manipulate bacterial pathways to enhance instead of disrupt the effect of antibiotics. They can investigate methods to disrupt biofilms as they are a reservoir for increased antibiotic resistance.

Concluding Remarks

Antibiotic-resistant bacteria have evolved due to mutations, mechanisms they implement to resist the effects of antibiotics, gene transfer methods through conjugation, transduction, and transformation, as well as developed tolerance to antibiotics through interactions with other microbes (Bottery et al., 2020; Christaki et al., 2019; MacLean & Millan, 2019). With these mechanisms and processes leading to genetic diversity, bacteria able to withstand antibiotics can survive, reproduce, and are favored by natural selection to spread antibiotic-resistant genes (Meek et al., 2015).

The evolution of antibiotic resistance makes it much more difficult to fight disease, putting a burden on us and various industries. It increases the health and economic burden on individuals and the cost of healthcare (Tacconelli & Pezzani, 2019). It has also increased hospital visits, mortality, and depleted funds to develop and research new strains of bacteria (Muteeb et al., 2023). Many pharmaceutical companies withdrew from antibiotic research and development due to low profits because of the consistent need to make a new antibiotic that has an effect that can diminish soon due to resistance (Muteeb et al., 2023).

This evidence calls for further experimental studies on alternative treatments to diseases other than the use of antibiotics to avoid the heavy reliance on their use (Christaki et al., 2019). This could be implemented by researching new therapies that are close to being as effective as antibiotics. One way to start researching alternative treatments is through an increase in government funding for pharmaceutical companies. Additional policies should be implemented to limit the use of antibiotics inside and outside of medicine to limit their spread (Tacconelli & Pezzani, 2019). For example, implementing policies in hospitals and clinics that ensure antibiotics are prescribed only when necessary can be achieved through further training of

healthcare professionals to ensure appropriate prescriptions for patients. Limiting the over-the-counter sale of antibiotics without a prescription could help prevent their overuse. In non-medical settings, decreasing antibiotics used in animal husbandry can also slow the spread of antibiotic resistance and can be a step in the right direction. Building on these strategies, other approaches in the manufacturing of antibiotics are essential to limit antibiotic resistance. Such examples include stricter regulations during the production process which control water and environmental contamination with byproducts of antibiotics to limit resistance. Furthermore, additional research on antibiotic-resistant pathways and disrupting biofilms could also pave the way for disruption methods to strengthen the effects of antibiotics on bacteria.

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Appendix

Glossary of Terms

Term	Definition
Bacteriophage	A virus that infects bacteria by attaching to the host bacterial cell and injecting its genetic material (Huemer et al., 2020).
Horizontal gene transfer	The movement of genetic information between unrelated bacteria that fuels antibiotic resistance and evolution (Burmeister, 2015).
Point mutation	When a single nucleotide base is substituted, deleted, or inserted in the DNA. (Jiang et al., 2021).
Prophage	A piece of the phage genome integrated into the bacterial host genome and replicated alongside it. (Chen et al., 2018).
Vertical gene transfer	The passage of plasmid from mother to daughter during cell division (Bethke et al., 2023).