Utilizing Phage Therapy to Overcome Antibiotic Resistance

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Utilizing Phage Therapy to Overcome Antibiotic Resistance

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Abstract:

Antibiotics are the primary, and arguably only, form of antimicrobial therapy against a pathogenic bacterial infection. With well over 250 million prescriptions written each year, it is clear that the health and well being of society is depending on the efficacy of antibiotic prescriptions. Based on their overarching mechanism of action, each class of antibiotics can be split into bactericidal or bacteriostatic categories, which either inhibits cellular growth and replication or decreases the viability of the bacterial cell. Although antibiotics have been proven to be successful in treating infections, things such as bacterial resistance mechanisms, adverse effects caused by antibiotics, and injudicious prescribing have led to a decrease in efficacy of the antibiotics we depend on and an increase in bacterial resistance. In order to combat this, a new form of antimicrobial therapy needs to be sought out. The use of bacteriophages in phage therapy could be a promising form of antimicrobial therapy. Backed by multiple studies, lytic bacteriophages can be used to target infections that are resistant to antibiotics, without targeting human cells or causing adverse effects, making them a promising candidate for the next antimicrobial therapy.

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1.1 Introduction on Antibiotics

Antibiotics: the foundation of treatment for bacterial infections. Often times, antibiotics are one of the common prescriptions we receive from a visit at the doctor's office or hospital when we are ill. In fact, antibiotics were such a popular course of treatment that 258.2 million antibiotic prescriptions were written in 2017, which reduces to 793 prescriptions per 100 people (Center for Disease Control and Prevention [CDC], 2019). Antibiotics are a cornerstone in modern medicine and have increased life expectancy while decreasing length of infection. They are used in the treatment of bacterial infections, preventative and post-operative care in surgeries, in chronic patients, and the list goes on (CDC, 2019). Only focusing on the fact that they are the solution that stands between us and a prolonged infection, we often take their abilities for granted and overlook what they are and their mechanism of action. The word antibiotic can be broken down in order to find its meaning. The prefix anti translates to against, opposing, or killing while the root word bio translates to life, therefore antibiotics are agents that kill living things or, in our case, pathogenic bacteria. However, antibiotics cannot be used to treat a viral infection due to the fact that the virus incorporates itself into a host cell in order to reproduce and do not possess the same machinery or characteristics, such as a cell wall, that these agents target. Antibiotics are used and prescribed to treat various bacterial infections within the body, and can be naturally occurring or synthesized from chemicals in a lab. The naturally occurring antibiotics, can come from fungus or soil bacteria, in which penicillin, tetracycline, streptomycin, and chloramphenicol is found (Clardy et al, 2009). Along with the different origins, these antimicrobial agents can also be identified as being broad-spectrum or narrow-spectrum based on the range of bacteria that they kill or inhibit (Clardy et al, 2009). Like the classifications imply, broad-spectrum antibiotics can affect multiple types of bacteria at once, including the normal flora that is present in the

body, while narrow-spectrum antibiotics are a more targeted antimicrobial agent that affects few types of bacteria.

1.2 Antibiotic classes and mechanism of action

Falling into two overarching classes, antibiotics can be identified as bacteriostatic or bactericidal based on their mechanism, or approach, to eliminating the pathogenic bacterial infection. Bacteriostatic antibiotics aim to stop the growth of the infection-causing pathogenic bacteria by inhibiting or stopping vital processes that contribute to the growth and replication of the organism. For example, the class of bacteriostatic antibiotics can inhibit protein production by interfering with ribosomal subunits and interfere with vital metabolic processes and enzyme activities (Loree and Lappin, 2020). Out of the general classes of antibiotics, bacteriostatic antibiotics include the macrolide, sulfonamide, tetracycline, linezolid, and lincosamide classes of antibiotics (Loree and Lappin, 2020). A summary of these antibiotics is illustrated in Figure 1.

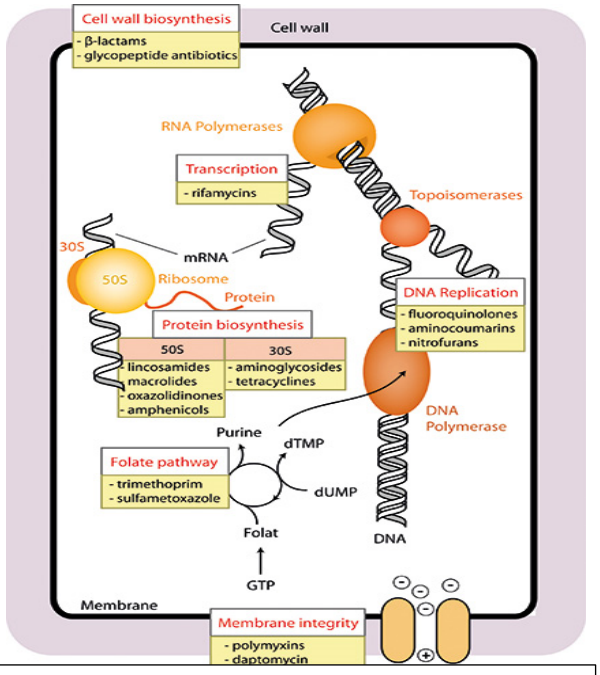

Figure 1: Antibiotic classes target different cellular mechanisms that are vital for growth and reproduction. Both bacteriostatic and bactericidal antibiotics interfere with various cellular mechanisms, affecting cell viability and growth. (Böttcher, 2015)

Macrolides treat gram-positive bacteria and atypical pathogens with broad-spectrum activity, include antibiotics such as erythromycin, clindamycin, and azithromycin, and inhibit protein synthesis in the elongation phase by selectively and reversibly binding with the 50S ribosomal subunit (Moore, 2020). Protein production is halted through the interaction of macrolides on the 23S ribosomal component, which lies within the larger 50s ribosomal subunit, which interferes with peptide bond formation in the peptidyltransferase center and blocks the path of the exiting polypeptide chain (Kanoh and Rubin, 2010). In antibiotic form, they generally contain large 12- to 16-membered glycosylated macrolactone rings that are attached to at least one deoxy sugar (Zin et al., 2020; Abdellatif et al., 2019). Although macrolides are naturally occurring, they can be created and altered via biosynthesis. The macrolide family consists of a rigid, organized structure that binds with high affinity to large protein binding pockets, and are characterized by having high membrane permeability, performing conformational flips in their solvent via hydrogen bonding and the polarity of the environment, and are not targeted by proteolytic degradation, all of which are recognized as positive drug properties (Zin et al., 2020). This class of antibiotics possess anti-inflammatory activity by decreasing the production of proinflammatory cytokines, such as interleukin 8 (IL-8), and is used to treat a multitude of respiratory infections (Kanoh and Rubin,2010). In addition to this, it also includes anticancer, immunomodulation, and antibiotic properties, which is why macrolides are also utilized in the treatment of *Chlamydia*, *Bordetella pertussis*, and *Helicobacter pylori* infections (Zin et al., 2020).

Also in the bacteriostatic category of antibiotics are sulfonamides. Sulfonamides, also commonly referred to as “sulfa drugs,” are antimicrobial agents that have broad-spectrum capabilities and can be utilized to target gram-positive and gram-negative bacterial

infections(Lin et al., 2020; Ophardt, 2003). A few examples of sulfa drugs are sulfadiazine, sulfisoxazole, and trimethoprim/sulfamethoxazole, all of which have an amino-phenylsulfonamide chemical structure (Lin et al., 2020). Sulfa drugs work by inhibiting the process of folic acid synthesis within the pathogenic bacterial cell. Folic acid is a vital nutrient to bacterial, as well as mammalian cells, but unlike mammalian cells, bacterial cells cannot uptake folic acid across their cell walls via diffusion or transport mechanisms, which requires the bacterial cell to possess the machinery to synthesize the folic acid that is imperative for its growth. The inhibition of folic acid synthesis is the result of competitive binding, due to the similar structure of the sulfa drug and para-aminobenzoic acid (PABA), which prevents PABA from being incorporated into folic acid (Ophardt, 2003). When the sulfa drug is substituted for the PABA that is normally used in the process of folic acid synthesis, the dihydropteroate synthase enzyme cannot carry out the reaction that combines glutamic acid with a PABA/pteridine product to produce folic acid, which results in the halt of production of folic acid within the bacterial cell (Ophardt, 2003; Yun et al., 2012). The sulfonamide class of antibiotics is effective at exploiting the aforementioned difference between mammalian and bacterial cells in order halt the growth of the targeted cells. This drug family is known for being highly stable, cheap to synthesize, and having broad-spectrum capabilities, and it prescribed to treat urinary tract infections, ear infections, *Escherichia coli* infections, infectious diarrhea, or to prevent bacterial infections (Lin et al., 2020; Wiedemann et al, 2014).

Similar to sulfonamides, tetracyclines also have broad-spectrum activity and are effective at treating both gram-positive and gram-negative bacteria, atypical, and spirochete bacteria (Loree and Lappin, 2020). The widespread activity of this antibiotic class, as well as the minimal adverse effects, has made them a commonly prescribed antibiotic agent in many doctor's offices

(Chopra and Roberts, 2001;Loree and Lappin, 2020). Antibiotics within the tetracycline family include, tetracycline, doxycycline, and minocycline, and their mechanism is focused on selective and reversible inhibition of protein synthesis via the 30S ribosomal subunit within the bacterial cell. Prior to binding with the 30S ribosomal subunit with high affinity, the tetracycline is transported across the outer membrane (of gram-negative bacteria) via porin channels OmpF and OmpC and later diffuses across the inner/cytoplasmic membrane (of gram-negative and gram-positive bacteria) in an electroneutral form (Chopra and Roberts, 2001). The tetracycline prevents the aminoacyl-tRNA from binding to the aminoacyl site (A-site) within the ribosome and inhibits the interaction of the codon-anticodon, which halts the production of the growing polypeptide chain and leads to the inhibition of protein synthesis (McMurry et al.,1980; Ophardt, 2003). The chemical structure of tetracycline, like other antibiotic classes, plays an important role in its antibiotic properties. Some of the chemical components of the tetracycline structure include a linear fused tetracyclic ring system, its naturally occurring stereochemical configurations, and possessing 4 dimethylamino groups on the cyclic ring system (Chopra and Roberts, 2001; Ophardt, 2003). The tetracycline class can be used for the treatment of acne, Lyme disease, *Mycoplasma* and *Rickettsia* infections, and for the prevention of malaria (Chopra and Roberts, 2001; Moore, 2020).

Like the previously mentioned macrolide class of antibiotics, the lincosamide family of antibiotics have a similar mechanism of action when it comes to killing pathogenic bacteria. Although there are multiple derivatives in the lincosamide class, the semi-synthetic chlorinated derivative, clindamycin, is the most commonly used in the clinical setting out of this antibiotic class (Spízek & Rezanka, 2017;Matzov et al., 2017). The mechanism of lincosamides is focused on the inhibition of protein synthesis by binding to the 50S bacterial ribosomal subunit and

causing an interference in the aminoacyl-tRNA complex and the addition of amino acids into the growing polypeptide chain, by directly inhibiting peptidyltransferase (Spízek & Rezanka, 2017;Matzov et al., 2017). In addition to this, the binding of clindamycin causes the release, or dissociation, of peptidyl-tRNAs from the ribosome and can interfere with the initiation phase of peptide chain formation, which halts the elongation process of protein production (Spízek & Rezanka, 2017). The chemical structure of the lincosamide class consists of moieties of amino acid and sugar that are linked by a peptide bond, specifically propylhygric acid and α-methylthiolincosamide, which is responsible for interaction with the nucleotides of the 23S ribosomal component within the larger 50S subunit (Matzov et al., 2017). This class is active against anaerobic bacteria, gram-positive and some gram-negative bacteria, and are known for having anti-staphylococcal and antistreptococcal activity (Spízek & Rezanka, 2017). Lincosamides and clindamycin can be utilized in the treatment of bone infections, abdominal/pelvic infection, malaria, toxoplasmosis, and staph and strep infections (Spízek & Rezanka, 2017; Loree and Lappin, 2020).

The linezolid class of antibiotics, similar to other antibiotic families that fall in the bacteriostatic category, has a mechanism of action that is focused on stopping protein production of the pathogenic bacterial cell by acting on its 50S ribosomal subunit. Unlike the macrolides and lincosamides that halt the process of protein production during the elongation step, linezolids prevent the production of proteins in the initiation step (Ament et al., 2017). By binding to the 30S and 50S subunits, the linezolid prevents the formation of the bacterial ribosome and the initiation complex, which results in no elongation process taking place and no polypeptide chain production (Hashemian et al., 2018; Loree and Lappin, 2020). Some features of the chemical structure that attribute to this antibiotic's activity include a 5-S configuration, N-aryl group, and

a 5-acylaminomethyl group (Hashemian et al., 2018). Linezolids have good activity against a variety of gram-positive organisms, especially those that have developed resistance to other antibiotic classes, such as drug-resistant *Staphylococcus*, *Pneumococcus*, *Enterococcus*, and *Streptococcus* (Ament et al., 2017;Hashemian et al., 2018). Because it is effective against drug-resistant infections like methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VREF), multiple type of hospital acquired pneumonia, and multidrug-resistant tuberculosis, linezolids are carefully prescribed and monitored to protect it against developing resistance (Hashemian et al., 2018;Loree and Lappin, 2020). This class has the ability to penetrate tissues across the body and can be utilized for infections of multiple sites.

On the other side of the spectrum are antibiotic classes that fall into the bactericidal category. The mechanism of action of bactericidal antibiotics is not focused on inhibiting the growth of the target bacteria, but on killing the infection-causing pathogenic bacteria. This category of antibiotics eliminates and kills the targeted bacteria by interfering with bacterial synthesis of proteins, impede DNA production by obstructing replication enzymes such as topoisomerase, and inhibit vital metabolic processes and enzymes (Kohanski et al., 2010). Included in the category of bactericidal antibiotics are aminoglycosides, quinolones and fluoroquinolones, beta-lactams, and glycopeptides.

The beta-lactams and glycopeptide classes of antibiotics affect the viability of the bacterial cell by interfering with cell wall biosynthesis, a vital process in cell growth and survival. Bacterial cell walls are composed of a peptidoglycan layer that lies outside of the plasma membrane, which is comprised of polysaccharide chains that are repeating units of N-acetylglucosamine linked to N-acetylmuramic acid covalently cross-linked to peptide chains (Kohanski et al., 2010).. The vital process of cross-linking the polysaccharide backbone to the

peptide chains is catalyzed out by an enzyme called transpeptidase (Kohanski et al., 2010). Due to the similar structure of Beta-lactams and peptidoglycan units, this antibiotic class targets the bacterial cell wall and inhibits synthesis by acting on penicillin-binding proteins (PBPs), which includes the aforementioned transpeptidase enzyme, within the cell membrane and binding to its active site (Ophardt, 2003). The irreversible binding of the antibiotic to the transpeptidase active site results in the inhibition of the enzymes ability to complete the cross-linking within the cell wall, resulting in the inhibition of cell wall synthesis that leads to cell death (Kohanski et al., 2010). The beta-lactam class includes penicillins and cephalosporins, and these antibiotics have activity against gram-positive and gram-negative bacteria (Moore, 2020). With a similar mode of action, the glycopeptide class of antibiotics also cause cellular death by interfering with the process of cell wall synthesis (Kohanski et al., 2010). Vancomycin, which is a part of the glycopeptide class, inhibits cell wall synthesis by binding to the peptidoglycan precursors needed for biosynthesis and interfere with the PBPs enzymatic activity, which leads to the halting of cell wall synthesis and death of the cell (Kohanski et al., 2010).

The classes of aminoglycosides and quinolones and fluoroquinolones are also included in the bactericidal category of antibiotics that kill rather than inhibit growth. The aminoglycoside class includes the antibiotics streptomycin and gentamycin, and these antibiotics have activity against gram-negative bacteria (Moore, 2020). Aminoglycosides affect the viability of the pathogenic bacteria through binding to the 16S component of the 30S ribosomal subunit, which induces a conformational change in the mRNA and charged aminoacyl-tRNA complex and causes a misreading of the genetic code (Kohanski et al., 2010). This conformational change and is mismatch of the tRNA results in the production of incorrect proteins or the interruption of protein synthesis as a whole (Kohanski et al., 2010). Lastly in the bactericidal category, the

quinolone and fluoroquinolone class has a mechanism unique to any that have been previously discussed. The quinolone class inhibits DNA replication within bacterial cells through targeting essential enzymes in the chromosome replication process (Kohanski et al., 2010). Specifically, the quinolone creates a complex with the cleaved DNA and the DNA gyrase and topoisomerase IV enzymes during the replication process, impacting the supercoiling of the DNA strands, and causing double stranded breaks in the DNA that ultimately leads to cell death (Gutierrez et al., 2018; Aminov, 2017). Whether antibiotics are recognized in the category of bactericidal or bacteriostatic, they both play vital roles in the health of the population.

1.3 The Need for Alternative Antimicrobial Therapy

Despite the fact that antibiotics still have the capability of preventing and treating many bacterial infections, there are many factors that point to the fact that it is imperative that treatment options are available in the near future. Things such as the adverse effects of antibiotics and antibiotic resistance are two large problems that have come to light due to the mis-use, over prescribing/excessive use, and the adaptive nature of the bacteria that we are fighting (APUA, n.d.). Bacterial resistance to antibiotics has been around since the discovery of antibiotics. According to the Center of Disease Control and Prevention, penicillin was discovered in 1941 and one year later, in 1942, antibiotic resistance had already appeared in penicillin-resistant *Staphylococcus aureus* (CDC, 2020). The same bacteria also became resistant to other forms of antibiotics. Methicillin, which is in the same antibiotic class as penicillin, was discovered in 1960, which was the same year that *Staphylococcus aureus* became resistant to it (CDC, 2020). Along the same trend, the CDC also highlights that vancomycin, part of the glycopeptide class, was discovered in the year of 1958 and *Staphylococcus aureus* later displayed resistance to this antibiotic in 2002 (CDC, 2020). This is just one example of the arms

race that we are in, and have been in for years, with the ever-evolving bacteria that continue to adapt and cause pathogenic infections throughout society. Bacteria have developed resistance mechanisms to every class of antibiotics that has been discussed to this point, which only further stresses the point that alternatives need to be available if we would like to have an advantage against the bacteria.

1.4 Bacterial Resistance Mechanisms

Not only is it important to know that pathogenic bacteria are continuously evolving against our medical advancements, it is important to know how the bacteria are acquiring resistance. Bacterial resistance can either be acquired or intrinsic, meaning that it can be genetic information that the bacteria naturally has or genetic information obtained by other means. An example of an intrinsic property that relates to antibiotic resistance is the cell wall of gram-negative bacteria; vancomycin is unable to penetrate the bacterial cell wall, so gram-negative bacteria are not affected by this antibiotic (Calhoun & Hall, 2020). Bacteria are also capable of gaining resistance that reduces an antibiotic's efficacy through spontaneous mutations or by obtaining resistance genes from other bacteria through mobile genetic elements (Calhoun & Hall, 2020).

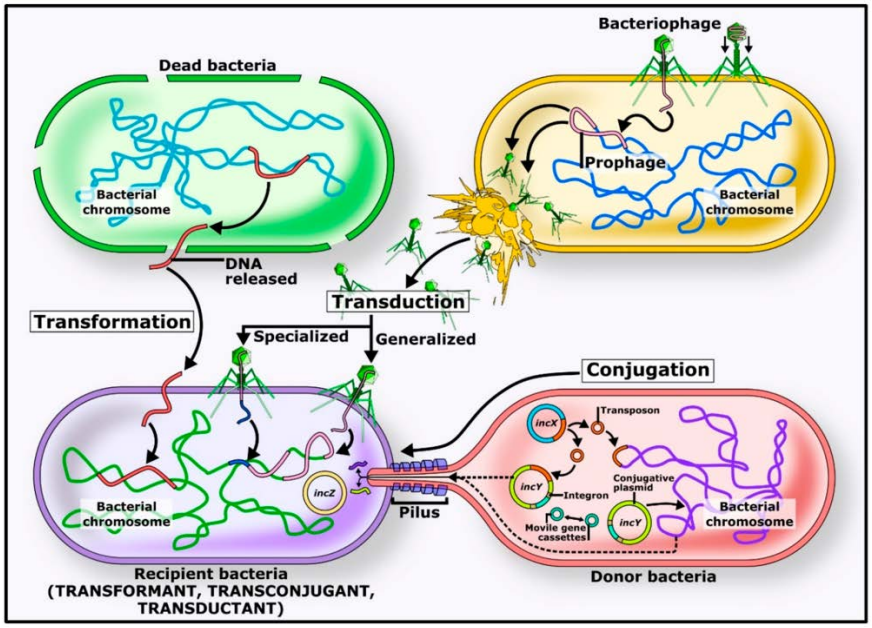

Figure 2: Mechanisms of horizontal gene transfer. Mobile genetic elements can be passed to other bacterial cells via transformation, transduction, or conjugation. This transfer can include antibiotic resistance genes or other genes that increase the fitness of the bacteria (Bello-López et al., 2019).

The mobile genetic elements can be passed in the form of a plasmid, transposon, integron, or passed to another bacterial cell through a bacteriophage as described in Figure 2 (Bello-López et al., 2019). The acquired resistance genes are taken up via horizontal transmission in three ways: conjugation, transduction, and transformation. Conjugation occurs when a genetic element, either a transposon or plasmid, is passed from one bacterial cell to another when connected via pilus (Giedraitienė et al., 2011). Transduction is when genetic material is transferred from a host cell to a bacterial cell by a bacteriophage. Finally, transformation occurs when the bacterial cell uptakes genetic information, typically in the form of a plasmid or a transposon, from its environment (Giedraitienė et al., 2011). The set of resistance genes that are passed from one bacteria to another can be group together and referred to as a "resistome," which includes genes that contribute to resistance from clinical and environmental bacteria, as well as intrinsic genes and protein-encoding genes that have the

ability to evolve into resistance genes (Bello-López et al., 2019). Due to the fact that the resistome contains a variety of resistance genes from multiple sources, it reveals that bacteria are acquiring antibiotic resistance capabilities from more fronts than we can combat. Any surviving bacteria after antibiotic treatment possesses the ability to pass on resistant properties, which means that there is a constant flow of resistance information between bacterial cells (Mayo Clinic, 2020). In the study conducted by Bello-López and colleagues, they determined that *Aeromonas* is capable of transferring genetic material through the process of conjugation to bacteria that are phylogenetically distant (2019). This is alarming due to the fact that genes contributing to antibiotic resistance can be transferred to and from bacteria that are not closely related. Evidence showing that resistance genes are spread to multiple bacteria contributes to the rising resistance against antibiotic activity. On top of this concern, bacteria can become to resistant to multiple classes of antibiotics, since cells can utilize the same resistance mechanism against multiple types of antibiotics.

A bacterial cell can possess a variety of resistance mechanisms against antibiotic treatment. Common resistance mechanisms include antibiotic modification, the expression of efflux pumps, and chemical modification of the antibiotic target. The spontaneous mutations in bacterial cells acquired through mistakes in DNA replication or repair, although rare, also have the ability to equip a bacterial cell with resistance mechanisms, and are only present in about one of every 10^6-10^8 microorganisms (Giedraitienė et al., 2011). Not only are pathogenic bacteria spontaneously mutating and gaining resistance against the antibiotics that we so heavily rely on as a society, they are also passing information to gain resistance mechanisms that have the ability to modify the antibiotics and their targets in order to render them useless. Antibiotic modifications are carried out by enzymes and lower the efficacy of the drug and has the ability to

completely inactive the drug (Giedraitienė et al., 2011). Examples of enzymatic resistance mechanisms are beta-lactamases and aminoglycoside-modifying enzymes that inactivate the antibiotic via hydrolysis or group transfer. Beta-lactamases inactivate antibiotics, such as penicillins, cephalosporins, and carbapenems, through hydrolysis of ester and amide bonds that are present in beta-lactam rings (Giedraitienė et al., 2011). Beta-lactamase classes A, C, and D are categorized aspenicilloylserine transferases that hydrolyze the beta-lactam ring in the active site by forming an acyl-serine intermediate that causes the inactivation the beta-lactam antibiotic (Schillaci et al., 2017). Additionally, this activity can be linked to the AmpC enzyme, that is chromosomally encoded by the ampC gene, that is present in all gram-negative bacteria and can be inhibitors of all penicillins and cephalosporins if present in high enough concentration (Mayers et al., 2017). This AmpC enzyme, also called serine beta-lactamases, is found in *Pseudomonas aeruginosa, Enterobacter* spp., and in the penicillinases present in *Staphylococcus aureus* (Giedraitienė et al., 2011). Furthermore, there is a class of beta-lactamases categorized as extended spectrum beta-lactamases (ESBL) that are produced by common gram-negative bacteria, and they show activity against the most potent classes, such as carbapenems, of the beta-lactam family of antibiotics (Schillaci et al., 2017). Bacterial strains that produce ESBLs include *Escherichia coli* and *Klebsiella pneumoniae* (Giedraitienė et al., 2011).The activity of ESBLs against carbapenems is especially alarming since this class is often reserved as last resort antibiotic. Aminoglycoside-modification enzymes (AMEs) are a group of transferase enzymes that modify and inactivate an antibiotic through group transfer reactions, and include acetyltransferases, phosphoryltransferases, and adenyltransferases to name a few (Giedraitienė et al., 2011). The AMEs block the activity of the drug and reduce the affinity by interfering with the binding of the antibiotic the 30S ribosomal subunit, and are found in *Staphylococcus aureus,*

Staphylococcus pneumoniae, and Enterococcus faecalis (Giedraitienė et al., 2011). Outside of inactivating the drug through enzymatic alteration, bacteria also evade antibiotics by modifying the antibiotic's target or expressing pumps to rid the drug from the bacterial cell.

Efflux pumps work by actively pumping the antibiotic from the cell, and can be found in both gram-positive and gram-negative bacteria (Schillaci et al., 2017). Many classes of antibiotics are expelled from the bacterial cell via efflux pumps, included tetracyclines due to *tet* genes, quinolones by NorA in *S. aureus*, and macrolides by Mef type pumps, and most efflux pumps have the capability to transport multiple drug classes(Giedraitienė et al., 2011). Pathogenic, and clinically important, bacteria that utilize these pumps include *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Escherichia coli* (Giedraitienė et al., 2011). Aside from pumping the drug out of the cell, another resistance mechanism is chemically modifying the antibiotic target. Antibiotics bind to their target with specificity, so small chemical changes can heavily influence the drug binding. *E. coli*, and other bacteria, have developed resistance to the sulfonamide class of antibiotics through a chromosomal mutation of the drug target, dihydropteroate synthetase enzyme, which reduces the affinity and allows the cell to regularly continue folic acid synthesis (Giedraitienė et al., 2011). There is also resistance to the quinolone class as a result of chemical modification of the drug target. Mutations of the amino acids in the *parC* and *gyrA* genes are responsible for the alteration of DNA gyrase and topoisomerase IV, the targets for the quinolone class, which causes a decrease in the affinity for the quinolone to its target and does not interrupt DNA replication (Giedraitienė et al., 2011). As resistance and resistance mechanisms increase, we lose our ability to fight the agents that threaten public health. Despite the fact that bacteria have and continue to develop numerous mechanisms of resistance against multiple classes of antibiotics, the bacteria are not acting alone.

1.5 Injudicious Distribution and Adverse Effects of Antibiotics

Society is also contributing to the rise of resistance in bacteria and decrease of the efficacy of the drugs that we rely on, and the injudicious prescribing of antibiotics is big contributing factor to the rise in resistance (APUA, n.d.). In addition to this, the continuing resistance to antibiotics and their adverse side effects are yet another call for an antibiotic alternative (Leekha et al., 2011). Although antibiotics are imperative in the treatment of pathogenic bacterial infections, many prescriptions are written when they are not necessary. According to the Center of Disease Control and Prevention (CDC), an estimated 47 million unnecessary antibiotic prescriptions are written in outpatient settings each year (CDC, 2019). The issue of patients being prescribed unnecessary antibiotics does not end in the outpatient setting but extents into other areas of healthcare, such as hospitals and nursing homes. On this topic, the CDC claims that "one-third of antibiotic prescriptions in hospitals involve potential prescribing problems such as giving an antibiotic without proper testing or evaluation, prescribing an antibiotic when it is not needed, or giving an antibiotic for too long" (CDC, 2019). The misuse and over-prescribing of antibiotics in the health care system may be due to empiric and prophylactic antibiotic therapy. Empiric therapy is when antibiotics are prescribed prior to obtaining the microbiological results and attempts to cover all pathogens that could be associated with the clinical presentations (Leekha et al., 2011). On the other hand, prophylactic therapy is used as a preventative measure for patients, such as those who are going into surgery, have traumatic injuries, or are immunocompromised (Leekha et al., 2011). Not only does the injudicious prescribing of antibiotics contribute to the problem of rising resistance in bacteria, it also affects the patients, and not only through its side effects. A study performed in 2010 "estimated that the cost of a hospital-acquired antibiotic-resistant infection results in 29% higher

cost-of-care and 24% longer hospital stay, compared to the same infection caused by an antibiotic-sensitive strain" (Mayers et al., 2017). In addition to this, antibiotics, like any other prescription, do not come without possible adverse effects. The adverse effects of antibiotics range from mild to severe. Mild side effects include things such as nausea, diarrhea, bloating, and abdominal pain, which the United Kingdom National Health Service estimates that around 1 in 10 people experience symptoms similar to those listed and about 1 in 15 people have an allergic reaction to their antibiotics (NHS, 2019). Contrary to the milder side effects, antibiotics can cause more severe effects that include, but are not limited to, toxic epidermal necrolysis, renal failure, QT prolongation, and even seizures (Granowitz and Brown, 2008;Wade & Williams, 2019). Along the same issue of concern, it has been stated that adverse reactions have occurred in 1 of 5 hospitalized patients (Calhoun & Hall, 2020). The adverse side effects associated with antibiotics, along with the over-prescription and rise of resistance further proves that an antibiotic alternative is needed in healthcare. The CDC estimates that a minimum of 2.8 million people each year have an antibiotic-resistant infection, and as a result 35,000 people die annually (CDC, 2020). With that staggering number, change is needed.

1.6 New Antimicrobial Therapeutic Option: Phage Therapy

With resistance rising, the trend of injudicious prescribing of antibiotics, and people being negatively affected by the antibiotics, a new therapy needs to be explored to rid the body of bacterial infections in order to put us back ahead in the arms race. An antibiotic alternative that could be our light at the end of the tunnel is the use of bacteriophages to aid in the fight against the pathogenic bacteria that are outsmarting our best forms of antibiotic therapy daily. Bacteriophages, which were discovered over a century ago, are parasitic viruses that attack bacteria with great specificity and are extremely abundant in nature (Kasman & Porter, 2019). In

addition to the previously mentioned characteristic, bacteriophages, otherwise referred to as phages, do not attack human cells or the normal flora within the body, do not cause adverse effects, and have the ability to adapt and evolve like bacterial cells. Like other viruses, phages need a host cell to replicate and survive as depicted in Figure 3 (Kotrikadze, 2018). Once a phage has reached its species-specific target cell, it attaches to the cellular surface via receptors, introduced a puncture to the cell surface, and injects its phage DNA into the bacterial cell (Kasman & Porter, 2019). The replication of the phage DNA that has been injected into the bacterial cell can either be lytic or lysogenic. If it is lytic replication, the host ribosomes are utilized to manufacture the proteins encoded by the phage DNA, which results in the accumulation of DNA and proteins needed to assemble another bacteriophage, as shown in Figure 3 (Kasman & Porter, 2019). The bacterial cell is lysed and newly formed bacteriophages escape to continue the process of infecting other bacterial cells. If replication is lysogenic, the phage DNA is either made into a plasmid or is integrated into the genetic material of the bacteria, which does not result in the killing of the cell and allows the genetic material to be passed on to daughter cells of the bacteria (Kasman & Porter, 2019). However, the replication can be converted back to the lytic cycle under certain conditions, which results in the death of the host cell.

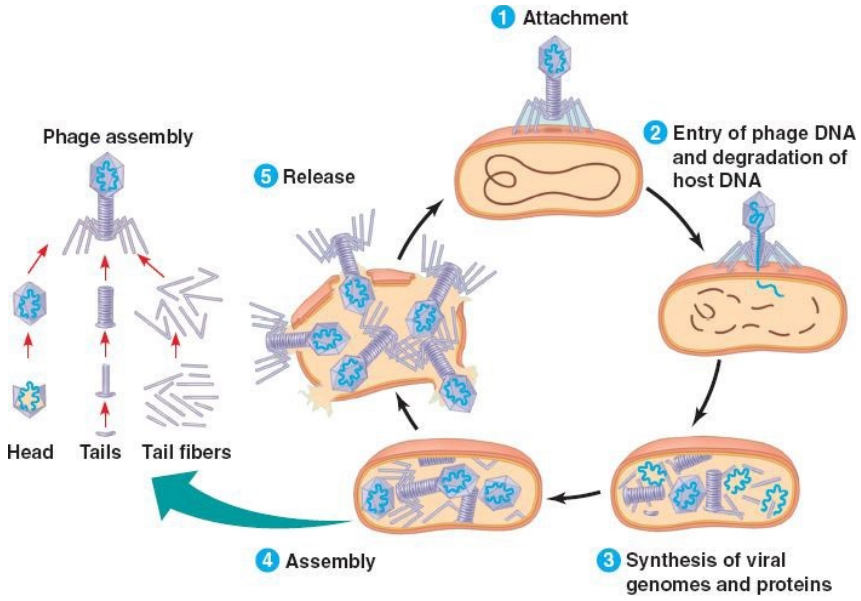

Figure 3: Mechanism of bacteriophages and lytic replication in bacterial cells (Kotrikadze, 2018).

Due to the characteristics of bacteriophages and their effect on bacterial cells, phage therapy is a promising approach to eliminating pathogenic bacterial infections, especially those that have proven to be multidrug- resistant. To support the speculation that phage therapy is a promising candidate as an antibiotic aid or alternative, several studies have been carried out to prove their efficacy in treating bacterial infections. In a study by Khairnar et al., phage therapy was used to treat ulcerative lesions that were present in catfish due to the multidrug-resistant bacteria *Pseudomonas aeruginosa*. Selected lytic phages were sterilely and directly applied to the lesions, in the concentration of 10^{10} per mL of bacteria, to 10 fish, while the other 10 were swabbed with phage-free diluents (Khairnar et al., 2013). Within 8-10 days, the group observed a reduction of the skin lesions of sevenfold, and the results were statistically significant with a 99% confidence interval and a p-value of less than 0.001 ($P>0.001$) (Khairnar et al., 2013). Positive results of phage therapy have not only been observed in non-human models, but in human trials as well (Aslam et al., 2019; Nir-Paz et al., 2019 & Tkhilaishvili et al., 2020).

Aslam and colleagues utilized phage therapy in the treatment of 3 lung transplant recipients that had acquired a lower respiratory infection, in conjunction with antibiotic therapy (Aslam et al., 2019). Two of the patients presented with a *Pseudomonas aeruginosa* infection, while the other presented with a *Burkholderia dolosa* infection. Patients 1 and 2 were both treated with the prefixed phage cocktail AB-PA01 for *P. aeruginosa* infections, and both clinically improved, eventually being discharged from the hospital at the end of phage therapy treatment (Aslam et al., 2019). Patient 3, who presented with a *B. dolosa* infection, positively responded to phage therapy twice, although eventually succumbing to a variety of complications that were believed to be non-phage related (Aslam et al., 2019). Some important things to note from the Aslam et al. study is that none of the patients experienced adverse effects from the phage therapy, the phage cocktails were able to be altered and personalized to eliminate specific clinical isolates, and two approaches to bacteriophage therapy, using a personalized cocktail verses pre-existing mixture, had positive outcomes (Aslam et al., 2019). In addition to this, Nir-Paz and colleagues touched on a case that also employed a combination of bacteriophage therapy and antibiotics and saw positive results. The patient of the Nir-Paz et al. case, who had a multidrug-resistant *Klebsiella pneumoniae* infection as well as an extensively drug-resistant *Acinetobacter baumannii* infection, was administered phage therapy as a last resort after failed surgical and antibiotic treatments (Nir-Paz et al., 2019). The phage therapy consisted of two phages that targeted both infection strains and were given in combination with antibiotics, which resulted in no side effects, wound closure, hospital discharge, and not positive cultures for either infection were present 8 months post-treatment (Nir-Paz et al., 2019). Similar to the previous case, Tkhilaishvili and colleagues also used personalized bacteriophage therapy in combination with antibiotic therapy to treat a relapsing periprosthetic joint infection caused by multi drug-

resistant *Pseudomonas aeruginosa* (Tkhilaishvili et al., 2020). The combination therapy of this case not only resulted in microbiological eradication of the infection but also supported that phage therapy can affect the viability of biofilm-embedded bacteria (Tkhilaishvili et al., 2020). Overall, this set of studies supports the use of bacteriophage therapy in the elimination of pathogenic bacterial infections, especially when antibiotics are not a suffice course of treatment. The results highlight the successes of both personalized and pre-formed phage cocktail approaches, the synergistic effect of bacteriophage therapy in combination with antibiotic therapy, the benefit of phage therapy causing no adverse effects on the patient, and the successful treatment of MDR infections.

1.7 Limitations and the Future of Phage Therapy

Although bacteriophage therapy has seen its successes, like any treatment there are concerns and limitations to overcome prior to it becoming available in the pharmacy or hospital setting. Some concerns may center around the effect and accumulation of phages in the body and the residual endotoxins released by the lysed cells (Schooley et al., 2017). Although multiple studies claim that phage therapy was well tolerated and the patient exhibited no side effects, the pharmacokinetic data have also indicated that phages do not accumulate after the course of therapy (Schooley et al., 2017; Aslam et al., 2019). The Aslam et al. study supports this by stating that no viable phages were found in the serum within 30 minutes of the next IV dose (Aslam et al., 2019). In addition, the study conducted by Schooley and colleagues reveals that phage clearance occurred within minutes in their human patient (Schooley et al., 2017). Furthermore, the Nir-Paz et al. case revealed that during the post-treatment follow-up period, which was 8 months in duration, revealed that no active phages were found in the blood, saliva, stool, or urine (Nir-Paz et al., 2019). In order to ensure that too many endotoxins are not released

in the body due to bacterial cell lysis, commercial assays can be completed, like in the Schooley et al. study, in order to find the amount of residual endotoxins that will be produced following administration of the phage or phage-cocktail (Schooley et al., 2017). Once the amount of endotoxin that is released from the bacteria by the phage, or phages, in question, the formula can be tweaked in order to adhere to the FDA endotoxin limit of 5EU/kg for humans.

In addition to the above concern, there are also limitations of phage therapy that need to be addressed before this is a viable antimicrobial approach that could be utilized in a pharmacy or hospital setting. Some limitations of phage therapy that needs to be addressed and overcome before it moves out of the trial phase and into clinical use are the rise of phage-resistant bacteria, the pharmacokinetics and pharmacodynamics of phage therapy, and the selection and proper preparation of the correct phages to combat the strain of bacteria that is causing the infection (Principi et al., 2019). In many studies, groups have focused more on the pass or fail outcome of their studies, rather than having a complete understanding of the mechanisms of the phages and the how and why the results came out as they did.

Bacteria have the ability to evolve and adapt phage-resistance mechanisms, like they have for antibiotics, which reveals why this is a limitation of phage therapy (Principi et al., 2019). Bacteria can evade infection by the phages through a variety of mechanisms such as the following: modification of the phage target or receptor, preventing phage adhesion through substance secretion, inhibiting DNA injection or phage replication, and by just simply hiding within the infected tissues to where the phages do not reach (Principi et al., 2019). Bacteria that have possessed these phage-resistant mechanisms are *Staphylococcus aureus, Escherichia coli,* and *Vibrio cholerae* (Principi et al., 2019). Although bacteria do have the ability to develop resistance mechanisms, phages also have the ability to evolve and counter-mutate the phage-

resistant mechanisms, and it has been stated that phages mutate at a faster rate, which would decrease the risk of the bacteria developing phage resistance (Parisien et al., 2007). In addition to this, Nilsson points out that there is a high rate of cells transitioning from being resistant to susceptible when the bacteria and phages are in a so-called arms race (Nilsson, 2019). Along these same lines, the specificity of the phage-resistant mechanisms that the bacteria have to develop makes it less likely for a broad-spectrum resistance mechanism to come about, such as efflux pumps or beta-lactamases (Schooley et al., 2017). One proposed solution is to use a phage cocktail, associate phage therapy with antibiotic therapy, or use a higher inoculum so the abundant bacteriophages kill the pathogens prior to them evolving resistance mechanisms (Principi et al., 2019). Phage cocktails may be used to target bacteria via different receptors, which in turn lowers the chance of resistance developing since the bacteria is being targeted at varying sites (Nilsson, 2019). The co-infection that is a result of using a phage cocktail may be synergistic, when the bacteria work together to clear an infection, or may have the potential of developing cross-resistance to other phages. The resistance developed when the bacteria is infected by one phage may result in resistance to another, or it may develop resistance to multiple phages that use the same receptor to attack the cell or affect a regulator of multiple receptors (Nilsson, 2019). Despite the fact that phages seem to have a leg up when it comes to evolving and counteracting the developments bacterial phage-resistance mechanisms, the interaction between phages and bacteria are not well understood and require more examination in order to fully know how to reduce or combat the developing resistance mechanisms in pathogenic bacteria.

Another present limitation of phage therapy is the pharmacokinetics and pharmacodynamics of phage therapy Although various studies have shown successful

applications of phage therapy, they lack the explanation why their methods were successful and mainly focus on the fact that there were results that indicated clinical improvement or infection clearing, which leaves out many parameters that affect the outcome (Nilsson, 2019). Due to the size of the phages, which are a million times larger than an antibiotic, the dose is limited to about 10^{13}-10^{14} PFU/mL but rarely achieved, especially after purification (Nilsson, 2019). The size of the phages affects the rates of transportation and uptake, and they are distributed throughout most organs, which results in a lower dose of phages reaching the site of infection than the initial amount that was given. Another limitation of therapy is that phages tend to bind to the bacterial debris left behind from lysed cells, which in turn prevents them from attaching to other viable bacteria (Nilsson, 2019). This may result in a lower efficacy of subsequent treatments, due to the fact that more debris will be present.

As far as pharmacodynamic limitations go, the efficacy of phage therapy is limited by the bacterial population the phages are targeting, specifically the phenotypic variation and physiological state of the cells (Nilsson, 2019). Bacterial cells are not uniform and do not have the same susceptibility. This is due to the fact that the bacterial cells are genetically different (Nilsson, 2019). Phenotypic variations include changes in cell structures caused by epigenetic regulation or phase shifting between cellular states and the replication capacity of the cell, which both contribute to the probability of infection (Nilsson, 2019).The physiological states of the cells, depending on available nutrients, can affect the number of phages that are produced, especially if the cell is in a dormant phase, however there are phages that are less dependent on the bacterial cell's nutritional state (Nilsson, 2019). To overcome the phenotypic variation that is present in bacteria populations and the fact that some phages that are dependent on the nutritional state of its host, phages must be carefully selected that combat these conditions.

Due to the varying nature of both bacterial cells, as well as bacteriophages, numerous phages are needed to compensate for the variations (Nilsson, 2019). This is where proper selection and preparation of phages for the pathogenic infection come into play. When selecting a phage, it must possess good antimicrobial activity against the target bacteria, does not code for genes that contribute to variation, virulence factors, toxins, or resistance (Nilsson, 2019). Genetic sequencing will allow for the selection of lytic phages over lysogenic phages, and could reveal if the phage possesses encoded genes result in the increased fitness of the bacterial cells. As far as phage preparation, as of now we currently lack common best practice and standardized purification methods (Nilsson, 2019).This results in varying purification, doses, and preparation between experiments, which makes it difficult to reproduce results or have everyone working in the same direction. By focusing efforts, rather than having varying approaches, phage treatment can become optimized and predictable.

As covered, there are many concerns and limitations regarding the utilization of phage therapy as an antimicrobial therapeutic option. Although some of these concerns and limitations can be mitigated through use of scientific techniques, there is much more work to do before phage therapy can be used in a hospital setting. As far as the future and overcoming these limitations, scientists have developed new mathematical models that more closely resemble a real phage therapy treatment, by taking pharmacodynamic problems into consideration (Nilsson, 2019). In addition to this, there is also a call for more animal and clinical trials that focus on the genetic and phenotypic changes that occur in the bacteria, as well as the phages, during therapy in order to get a better understanding of the how and why the outcome occurred (Nilsson, 2019). A call for phage study and collection has also been made, in order to increase the knowledge about the phages in our environment and their activity against bacteria. An example of a phage

collecting initiative is the Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science Program (SEA-PHAGE). This initiative is aimed at undergraduate students who carry out everything from phage collection to purification to DNA sequencing and even discovering the host range of their isolated bacteriophage (SEA-PHAGE, n.d.). With this information, phages are characterized and can be categorized based on their target, making it easier for phage selection when it comes to treating pathogenic infections in the future. This library of phages makes future studies easier, especially when they run into resistance problems and need an additional phage to add to their cocktail to combat the resistant bacteria. With in-depth understanding of the pharmacokinetic and pharmacodynamic properties of phage therapy, in addition to proper established methods and an available source of characterized phages, the number of successful phage treatments would increase and the probability that phage therapy would be closer to being utilized in a pharmacy setting would be higher.

1.5 Conclusions

It is clear that antibiotics will not be a substantial form of therapy forever. With continuously evolving bacteria, the antibiotics that have served as a cornerstone for fighting bacterial infections for many decades are slowing become more ineffective. Something must be done to preserve the efficacy of the antibiotics that we have left if we want to continue to fight pathogenic bacteria. This is where bacteriophages, and phage therapy, come into play. The target-specific lytic phages are a promising alternative or aid to the antibiotics we have now, due to the fact that they do not attack human cells, have the ability to evolve like their bacterial targets, have proven successful in fighting multidrug-resistant and extensively drug-resistant infections, and are extremely abundant in the environment. These phages can be used in a personalized approach that targets a specific strain or in combination with other phages, referred

to as a phage-cocktail, in order to have broader activity. Whether bacteriophages are used in combination with antibiotic therapy in order to get a synergistic effect, or on their own, why not recruit these natural-born killers into our arsenal against the pathogenic bacterial infections? We need to turn the tide of this arms race, and phages are a step in the right direction.

REFERENCES:

Abdellatif, M., Ghozy, S., Kamel, M. G., Elawady, S. S., Ghorab, M. M. E., Attia, A. W., … Huy, N. T. (2019). Association between exposure to macrolides and the development of infantile hypertrophic pyloric stenosis: a systematic review and meta-analysis. *European Journal of Pediatrics*, *178*, 301–314. doi: 10.1007/s00431-018-3287-7

Alliance for the Prudent Use of Antibiotics (APUA). About Resistance. (n.d.). Retrieved from https://apua.org/about-resistance

Ament, P., Jamshed, N., & Horne, J. (2002). Linezolid: Its Role in the Treatment of Gram-Positive, Drug-Resistant Bacterial Infections. *American Family Physician*, *65*(4), 663–670. doi: 15;65(4):663-671.

Aminov, R. (2017). History of antimicrobial drug discovery: Major classes and health impact. *Biochemical Pharmacology*, *133*, 4–19. doi: 10.1016/j.bcp.2016.10.001

Böttcher, T. (2015, February 13). Antibiotic Resistance: Facing the Challenges of Bacterial Infections. Retrieved from https://analyticalscience.wiley.com/do/10.1002/gitlab.12975/full/

Bello-López, J. M., Cabrero-Martínez, O. A., Ibáñez-Cervantes, G., Hernández-Cortez, C., Pelcastre-Rodríguez, L. I., Gonzalez-Avila, L. U., & Castro-Escarpulli, G. (2019). Horizontal Gene Transfer and Its Association with Antibiotic Resistance in the Genus Aeromonas spp. *Microorganisms*, *7*(9), 363. doi: 10.3390/microorganisms7090363

Calhoun, C., & Hall, G. (2020, February 17). Antibiotics. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK535443/

Center for Disease Control and Prevention.(2020, March 13). About Antibiotic Resistance. Retrieved from https://www.cdc.gov/drugresistance/about.html

Center for Disease Control and Prevention. (2019, August 8). Antibiotic Use in Outpatient Settings, 2017. Retrieved from https://www.cdc.gov/antibiotic-use/stewardship-report/outpatient.html

Center for Disease Control and Prevention. (2019, October 22). Outpatient Antibiotic Prescriptions - United States, 2017. Retrieved from https://www.cdc.gov/antibiotic-use/community/programs-measurement/state-local-activities/outpatient-antibiotic-prescriptions-US-2017.html

Chopra, I., & Roberts, M. (2001). Tetracycline Antibiotics: Mode of Action, Applications, Molecular Biology, and Epidemiology of Bacterial Resistance. *Microbiology and Molecular Biology Reviews*, *65*(2), 232–260. doi: 10.1128/mmbr.65.2.232-260.2001

Clardy, J., Fischbach, M. A., & Currie, C. R. (2009). The natural history of antibiotics. *Current biology : CB*, *19*(11), R437–R441. https://doi.org/10.1016/j.cub.2009.04.001

Giedraitienė, A., Vitkauskienė, A., Naginienė, R., & Pavilonis, A. (2011). Antibiotic Resistance Mechanisms of Clinically Important Bacteria. *Medicina*, *47*(3), 19. Doi:10.3390/medicina47030019

Granowitz, E. V., & Brown, R. B. (2008). Antibiotic Adverse Reactions and Drug Interactions. *Critical Care Clinics*, *24*(2), 421–442. doi: 10.1016/j.ccc.2007.12.011

Gutierrez, A., Stokes, J., & Matic, I. (2018). Our Evolving Understanding of the Mechanism of Quinolones. *Antibiotics*, *7*(2), 32. doi: 10.3390/antibiotics7020032

Hashemian, S. M., Farhadi, T., & Ganjparvar, M. (2018). Linezolid: a review of its properties, function, and use in critical care. *Drug Design, Development and Therapy*, *12*, 1759–1767. doi: 10.2147/dddt.s164515

Kanoh, S., & Rubin, B. K. (2010). Mechanisms of Action and Clinical Application of Macrolides as Immunomodulatory Medications. *Clinical Microbiology Reviews*, *23*(3), 590–615. doi: 10.1128/cmr.00078-09

Kasman, L. M., & Porter, L. D. (2019, August 12). Bacteriophages. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK493185/

Kohanski, M. A., Dwyer, D. J., & Collins, J. J. (2010). How antibiotics kill bacteria: from targets to networks. *Nature Reviews Microbiology*, *8*(6), 423–435. doi: 10.1038/nrmicro2333

Kotrikadze, L. (2018, June 19). Regulating Bacteriophages: The First Steps into the Post-Antibiotic Era. Retrieved from http://www.biopharma-excellence.com/news/2018/7/17/regulating-bacteriophages-the-first-steps-into-the-post-antibiotic-era

Leekha, S., Terrell, C. L., & Edson, R. S. (2011). General Principles of Antimicrobial Therapy. *Mayo Clinic Proceedings*, *86*(2), 156–167. doi: 10.4065/mcp.2010.0639

Lin, Z.-Z., Li, L., Fu, G.-Y., Lai, Z.-Z., Peng, A.-H., & Huang, Z.-Y. (2020). Molecularly imprinted polymer-based photonic crystal sensor array for the discrimination of sulfonamides. *Analytica Chimica Acta*, *1101*, 32–40. doi: 10.1016/j.aca.2019.12.032

Loree, J., & Lappin, S. L. (2020). *Bacteriostatic Antibiotics*. Treasure Island, FL: StatPearls Publishing. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK547678/

Matzov, D., Eyal, Z., Benhamou, R. I., Shalev-Benami, M., Halfon, Y., Krupkin, M., … Yonath, A. (2017). Structural insights of lincosamides targeting the ribosome of Staphylococcus aureus. *Nucleic Acids Research*, *45*(17), 10284–10292. doi: 10.1093/nar/gkx658

Mayers, D. L., Sobel, J. D., Ouellette, M., Kaye, K. S., & Marchaim, D. (2017). *Antimicrobial drug resistance*(2nd ed.). Springer. doi: 10.1007/978-3-319-46718-4

Mayo Clinic. (2020, February 15). Antibiotics: Are you misusing them? Retrieved from https://www.mayoclinic.org/healthy-lifestyle/consumer-health/in-depth/antibiotics/art-20045720

McMurry, L., Petrucci, R. E., & Levy, S. B. (1980). Active efflux of tetracycline encoded by four genetically different tetracycline resistance determinants in Escherichia coli. *Proceedings of the National Academy of Sciences*, *77*(7), 3974–3977. doi: 10.1073/pnas.77.7.3974

Moore, D. (2020, January 20). Antibiotic Classification & Mechanism. Retrieved from https://www.orthobullets.com/basic-science/9059/antibiotic-classification-and-mechanism

Nilsson, A. S. (2019). Pharmacological limitations of phage therapy. *Upsala Journal of Medical Sciences*, *124*(4), 218–227. doi: 10.1080/03009734.2019.1688433

Ophardt, C. E. (2003). Antibacterial Agents - Sulfa Drugs. In *Virtual ChemBook*. Retrieved from http://chemistry.elmhurst.edu/vchembook/653sulfa.html

Ophardt, C. E., & Ophardt, C. E. (2003). Other Antibiotics. In *Virtual ChemBook*. Retrieved from http://chemistry.elmhurst.edu/vchembook/654antibiotic.html

Parisien, A., Allain, B., Zhang, J., Mandeville, R., & Lan, C. (2007). Novel alternatives to antibiotics: bacteriophages, bacterial cell wall hydrolases, and antimicrobial peptides. *Journal of Applied Microbiology*, *104*, 1–13. doi: 10.1111/j.1365-2672.2007.03498.x

Principi, N., Silvestri, E., & Esposito, S. (2019). Advantages and Limitations of Bacteriophages for the Treatment of Bacterial Infections. *Frontiers in Pharmacology*, *10*. doi: 10.3389/fphar.2019.00513

Schillaci, D., Spanò, V., Parrino, B., Carbone, A., Montalbano, A., Barraja, P., … Cascioferro, S. (2017). Pharmaceutical Approaches to Target Antibiotic Resistance Mechanisms. *Journal of Medicinal Chemistry*, *60*(20), 8268–8297. doi: 10.1021/acs.jmedchem.7b00215

Schooley, R. T., Biswas, B., Gill, J. J., Hernandez-Morales, A., Lancaster, J., Lessor, L., … Hamilton, T. (2017). Development and Use of Personalized Bacteriophage-Based Therapeutic Cocktails To Treat a Patient with a Disseminated Resistant Acinetobacter baumannii Infection. *Antimicrobial Agents and Chemotherapy*, *61*(10). doi: 10.1128/aac.00954-17

SEA-PHAGES: Home. (n.d.). Retrieved from https://seaphages.org/

Spízek, J., & Rezanka, T. (2017). Lincosamides: Chemical structure, biosynthesis, mechanism of action, resistance, and applications. *Biochemical Pharmacology*, *133*, 20–28. doi: 10.1016/j.bcp.2016.12.001

United Kingdom National Health Service. (2019, May 23). Side Effects-Antibiotics. Retrieved from https://www.nhs.uk/conditions/antibiotics/side-effects/

Wade, S., & Williams, M. (2019). Antibiotic side-effects: from the anticipated to the bizarre. *Prescriber*, *30*(11), 16–21. doi: 10.1002/psb.1801

Wiedemann, B., Heisig, A., & Heisig, P. (2014). Uncomplicated Urinary Tract Infections and Antibiotic Resistance—Epidemiological and Mechanistic Aspects. *Antibiotics*, *3*(3), 341–352. doi: 10.3390/antibiotics3030341

Yun, M.-K., Wu, Y., Li, Z., Zhao, Y., Waddell, M. B., Ferreira, A. M., … White, S. W. (2012). Catalysis and Sulfa Drug Resistance in Dihydropteroate Synthase. *Science*, *335*(6072), 1110–1114. doi: 10.1126/science.1214641

Zin, P. P. K., Williams, G., & Fourches, D. (2020). SIME: synthetic insight-based macrolide enumerator to generate the V1B library of 1 billion macrolides. *Journal of Cheminformatics*, *12*(1). doi: 10.1186/s13321-020-00427-6