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Antibiotics: A Vital Aspect of Medicine Turned Public Health Concern

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Antibiotics: A Vital Aspect of Medicine Turned Public Health Concern

By

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An Honors Thesis Submitted in Partial Fulfillment
of the Requirements for Graduation from the
Western Oregon University Honors Program

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ABSTRACT

Since their discovery, antibiotics have been a critical asset to medicine. They are responsible for saving lives and treating infections that could have been damaging or life-threatening in their absence. In short, we have become dependent on antibiotics. Now, however, antibiotic resistance in microbial infections has caused antibiotics, our “miracle drugs”, to diminish in their effectiveness. This, in turn, has the potential to greatly impact the health of the entire human population. Aspiring to become a doctor concerned with both the health of the general public and myself, I seek to understand this issue in its entirety and aim to produce a resource so that others can do the same. Through the use of scientific articles and other scholarly materials, I will examine, analyze, and compile research to determine what has caused antibiotic resistance, and what can be done to prevent it from worsening. It is crucial that we understand the seriousness of this issue and the role it plays in our lives. By creating a resource that thoroughly explains the different aspects of antibiotic resistance, ranging from its origins and current antibiotic resistant infections to strategies that may help stop it, I hope to provide an opportunity for the general public to become aware and well-informed about this issue.

I. INTRODUCTION

In the middle of the night a person awakens with a fever, by morning they feel worse, and by afternoon they are sitting in their doctor's waiting room. Upon examination, the doctor makes note of certain symptoms that encourage her to run some tests. The results of those tests confirm her fears; the patient has a bacterial infection. The patient, however, isn't worried; they hear about infections all the time as well as the antibiotics to treat them. Who really dies of a simple infection nowadays, anyway? The patient leaves the doctor, unhappy about the diagnosis, but confident that the prescribed antibiotic they now have will cure their ailment.

A BRIEF HISTORY OF ANTIBIOTIC DISCOVERY

It seems that in today's society, antimicrobial agents have become commonplace, but our knowledge about these agents, especially antibiotics, is relatively recent. The road to understanding and employing antibiotics has been arduous, and the success over the years is the result of exhausting work and countless failures. People have been searching for methods to treat ailments for millennia. This quest goes back to rudimentary treatments ranging from mud, herbs, other plants and even magic. Although some ideas are more far-fetched than others, there have been remedies employed over the years that led to a greater understanding. Early people relied on plants to cure ailments and treat

wounds. An idea not entirely misguided considering that more than 2,500 plants contain antimicrobial properties [23]. Specific plants such as *Hypericum perforatum*, more commonly known as St. John's Wort, and *Symphytum officinal* (a.k.a. Common Comfrey) also have actual antibacterial qualities [23]. In addition to making use of the local flora, other substances were discovered to be beneficial as treatments. Mercury was successfully employed by the Egyptians as an antibacterial agent [23] and it was also thought that the red soils found in Jordan, Africa were capable of treating infections similar to our modern day antibiotics [2].

These primitive treatment strategies were discovered during a time known as the pre-antibiotic era and were not only valuable for early cultures, but also served to introduce remedies that would later become useful in the antibiotic era. Jordan's red soil, was one such example, which helped to lay the groundwork for scientific advancements and further understanding [2].

As decades passed, and the wealth of knowledge increased, treatments grew more advanced and a search for new ones ensued. What came to be known as the aforementioned "Antibiotic Era," began with the work of two individuals. The first was Paul Ehrlich, a German chemist who aimed to create a chemical compound that could leave healthy, body cells intact while eliminating bacteria. This chemical compound was often referred to as a "magic bullet" [2,19]. His

work targeted the bacterium, *Treponema pallidum*, which causes Syphilis, a sexually transmitted disease (STD). Along with a research team consisting of Alfred Bertheim and Sahachiro Hata, Ehrlich tested one compound after another on laboratory rabbits infected with the STD of interest [2]. Despite numerous failed attempts, they successfully discovered a chemical compound that could target the bacterium and leave the body's cells unaffected. This 1909 discovery occurred after 605 failed chemical attempts and was justly coined "Compound 606," although it was officially named arsphenamine and was marketed under the trade name Salvarsan™ [2,19]. It became the main treatment for Syphilis and was the most prescribed drug on the market at that time [2].

Arsphenamine dominated the pharmacy until the introduction of penicillin in the 1940s. What would come to be the first antibiotic began with a mere observation of mold. In 1928, Alexander Fleming examined a laboratory culture of *Staphylococcus* bacteria and realized the growth of the bacteria was hindered by the presence of a mold containment [19]. The unexpected containment was *Penicillium notatum*. This moment of sheer chance spurred further investigation into the antibiotic properties associated with the mold. Fleming knew that the mold itself could be administered in a non-harmful form to living organisms, and coupled with the fact that it could inhibit bacterial growth, led Fleming to pursue a way to purify and stabilize the responsible compound. This task proved

challenging and unsuccessful for over a decade. However, in 1940 the answers had finally been revealed. A team of biochemists, Howard Walter Florey and Ernst Boris Chain, were able to purify penicillin and published their results [2,19]. Using their methods, it was possible to produce and clinically test penicillin. The mass production and successful human administration of penicillin would follow two years later.

Both Paul Ehrlich and Alexander Fleming made noteworthy contributions to medicine and led the world into the antibiotic era. Although their overall drug discoveries are valuable, they also assisted future generations through the development of new laboratory strategies. Ehrlich's work introduced both a treatment for Syphilis and a new protocol for drug research. The strategies he and his team employed during the research and development of arsphenamine created a framework that was eagerly adopted by pharmaceutical companies [2]. Following Ehrlich's methods, countless new antimicrobial agents, along with other drugs, were created. Likewise, while making the observation that would lead to the development of penicillin, he also was unintentionally discovering a new method for testing the effectiveness of antibiotics and antibiotic-producing organisms [2]. Prior to Fleming, any testing of antimicrobials was accomplished only through the use of a large quantity of resources. However, observing the results of products on plates of pathogenic bacteria, as Fleming first had, was

more efficient and required fewer materials. In this way, Ehrlich and Fleming provided valuable drug discoveries and changed the protocols employed in the laboratory. As technology and knowledge have advanced since Ehrlich and Fleming's contributions, additional antimicrobial compounds have been discovered or created. Nevertheless, the antibiotic industry was impacted by both the early uses of naturally occurring antimicrobials and the efforts of Ehrlich and Fleming.

LABORATORY FINDINGS

The bacterial domain is complex; there are not merely a handful of bacterial strains, but rather, multitudes to contend with. As described in the Literature Review below, bacteria can be categorized according to their shape. However, they can also be grouped based on cell wall chemical composition; three categories are typically recognized. Gram-positive bacteria have cells walls containing a thick portion of a sugar and amino acid polymer, peptidoglycan. Gram-negative bacteria also have peptidoglycan in their cell walls, but the layer is much thinner compared to their gram-positive relatives. This category of bacteria also contains an outer membrane called lipopolysaccharide (LPS) [49]. Additionally, the third category completely lack walls and are known as "cell wall-deficient" (CWD). As the name suggest, these species of bacteria are unable to produce cell walls. [19].

A common and valuable laboratory test called the Gram Stain is used to determine differences in cell wall composition. Developed by Hans Christian Gram in 1883, the Gram stain allows differentiation between gram-positive and gram-negative bacteria [19]. The procedure includes treatment with a crystal violet solution followed by an iodine solution that indicates the presence of gram-positive bacteria. The next steps are an alcohol rinse followed by a safranin counterstain to test for gram-negative bacteria [19]. If gram-positive bacteria are present, they will remain violet in color, while gram-negative bacteria will appear red. Distinguishing between gram-positive and negative bacteria is beneficial in identifying species of bacteria.

Understanding cell wall differences of bacteria has contributed to the development of antibiotics, as well. Antibiotics can be classified as either broad or narrow spectrum. This differentiation in antibiotics refers to the types of bacteria certain types of antibiotics are effective against. Broad spectrum antibiotics are able to hinder both gram-positive and negative bacteria, while narrow spectrum antibiotics are only able to target one or the other [19].

In an effort to better understand these differences, as well as gain firsthand experience in laboratory procedures and the efficacy of antibiotics, I examined the effects of four antibiotics on three bacterial species in a laboratory setting. The three species were *Serratia marcescens*, *Micrococcus luteus*, and

Citrobacter freundii, *S. marcescens* [68] and *C. freundii* [10,30] are gram-negative bacteria, while *M. luteus* is gram-positive [31]. These three species were each exposed to the same four drugs: penicillin, streptomycin, oxacillin, and sulfamethoxazole trimethoprim. The first three are antibiotics, while the fourth is a synthetic, man-made drug. Streptomycin and sulfamethoxazole trimethoprim are both broad spectrum drugs [19,48]. Streptomycin is an aminoglycoside that damages bacteria by interfering with protein synthesis [19,77]. Sulfamethoxazole trimethoprim or “sulfa” is a member of the sulfonamide category of antibacterial agents. Penicillin and oxacillin are both narrow spectrum antibiotics. Penicillin is specifically a β -lactam antibiotic that disrupts cell wall synthesis in bacteria. Similar to penicillin, oxacillin falls into the β -lactam class of drugs and is closely related to methicillin [49,63].

Based on the spectra of antibacterial drugs in conjunction with cell wall composition of the bacteria, the effects of each antibiotic can be predicted. Since streptomycin, and sulfa are broad-spectrum drugs, they potentially could inhibit growth of all three bacterial species. Oxacillin, as well, should have little effect on *S. marcescens* due to its similarity with penicillin. *C. freundii* is commonly resistant to penicillin and oxacillin [10]. Nothing in literature suggested that *M. luteus* would be unresponsive to any of the four antibiotics. This information led me to predict that *S. marcescens* would only be inhibited by sulfa, *M. luteus*

should be affected by all four drugs, and *C. freundii* should be responsive to streptomycin and sulfa.

To test these predictions, 18 plates of nutrient agar were prepared. Nutrient agar is a non-selective medium that contains beef extract and allows many kinds of microbes to grow. The initial 18 plates were separated into three groups of six. Each group was double inoculated with one of the three bacterial species using a spreader. Common inoculation and sterilization procedures were implemented. Following preparation and inoculation, one antibiotic disk previously saturated with the specific antibacterial drug was applied to a “sector” of the plate. Each plate received a total of four antibiotic disks, one per sector for a total of four sectors (Fig. 1). The plates were then incubated for 48 hours, after which, each zone of inhibition was measured. A zone of inhibition is an area immediately surrounding an antibacterial disk where no bacterial growth occurs. A zone of inhibition indicates the susceptibility of that microbe to the specific drug [49].

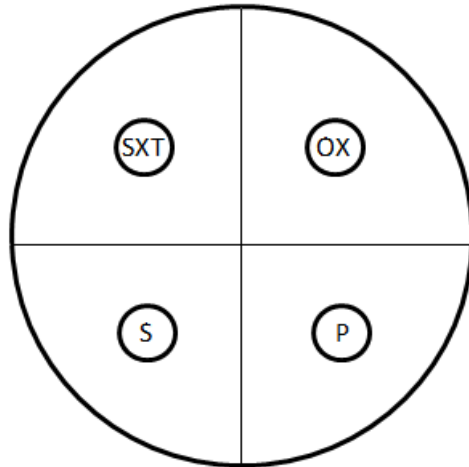


Figure 1: Drug disk setup on nutrient agar plates. The horizontal and vertical lines divide the plate into four “sectors.” Each sector contains one drug disk: streptomycin (SXT), oxacillin (OX), sulfa (S), and penicillin (P).

Two additional plates were prepared to test for the presence of two gram-positive bacteria often found on skin, *Staphylococcus epidermidis* and *Staphylococcus aureus*. Rather than the non-selective agar previously used, these bacteria were cultured on plates containing mannitol salt. Mannitol salt is selective because it normally inhibits gram-negative bacteria. Swab samples taken from inside my nostrils and between my toes were used to inoculate the plates. The plates were then incubated for 48 hours.

The results of the antibacterial drug experiment aligned closely with the predictions previously stated. All zones of inhibition were measured to determine the efficacy of each antibiotic on each bacterial species. *S. marcescens* was only inhibited by sulfa and maintained an average 3 mm zone of inhibition for all six plates (Fig. 2). *M. luteus* was inhibited by all four antibiotics (Fig. 2). For this

particular bacterium, the average zone of inhibition for streptomycin was 7.5 mm, oxacillin was 11 mm, sulfa was 5.8 mm, and penicillin was 18.7 mm. *C. fruendii* was affected by streptomycin and sulfa, but not by oxacillin or penicillin (Fig. 2). Streptomycin's zone of inhibition was 8.8 mm and sulfa's was 4.7 mm. The two mannitol salt plates showed growth of more than 300 colonies of *S. epidermidis*, but no *S. aureus* growth occurred. These results indicate that I harbor *S. epidermidis* colonies on my skin, but not *S. aureus*.

PLATE #	<i>S. marcescens</i> (mm)				<i>M. luteus</i> (mm)				<i>C. fruendii</i> (mm)			
	SXT	OX	S	P	SXT	OX	S	P	SXT	OX	S	P
1	0	0	3	0	7	11	5	20	9	0	4	0
2	0	0	3	0	9	11	5	18	9	0	6	0
3	0	0	3	0	7	11	7	19	9	0	5	0
4	0	0	3	0	7	10	6	18	8	0	5	0
5	0	0	3	0	8	12	6	20	9	0	4	0
6	0	0	3	0	7	11	6	17	9	0	4	0

Figure 2: Measurements, in millimeters, for the zones of inhibition observed on three sets of 6 agar plates inoculated with *S. marcescens*, *M. luteus*, and *C. fruendii*, respectively. Legend: streptomycin (SXT), oxacillin (OX), sulfa (S), and penicillin (P) disks.

The results of this study supported previous research as well as my hypotheses. While this study did not contribute to the discovery of any new information, it did serve as an important personal educational and informational lesson into the effects of antibacterial drugs on bacteria. Understanding the different categories of bacteria based on cell wall differences, as well as being introduced to antibiotic spectra and laboratory procedures for bacterial identification and testing, provided me with a firm foundation to begin understanding the issue of antibiotic resistance.

II. LITERATURE REVIEW

Antibiotic resistance is a term used in situations where an antibiotic, when applied to a microbe, will have either no, or a diminished effect on the microbe. The phrase, “antibiotic resistance,” is frequently used by, and has become very familiar to, today’s society. In order to truly understand antibiotic resistance, it is important to be aware of the history of antibiotics. In order to glean a greater understanding of this multi-faceted issue, scientific literature published prior to 2000 has been examined. Throughout this collection of sources, reoccurring topics emerged that serve as the basis for developing a sound foundation about antibiotic resistance. A majority of sources explained the mechanisms by which antibiotic resistance develops, whereas others focused on the uses of antibiotics and the impacts their existence has created. I consulted additional references by

Chee-Sanford, Aminov, Krapac, Garrigues-Jeanjean, and Mackie [9], Cohen and Tauxe [11], Falkiner [22], Greko [27], Kumar, Gupta, Chander, and Singh [35], McManus, Stockwell, Sundin and Jones [54], Mølbak et al. [55], Summers [79], van den Bogaard and Stobberingh [81], Voss, Loeffen, Bakker, Klaassen, and Wulf [83], and Wilkins [87]. I also read two articles by Levy [39,40] and two articles by Levy, FitsGerald, and Macone [42,43]. I consulted all of these references in order to develop a broader understanding of the issues surrounding antimicrobial resistance as I embarked on my research

The discovery and development of antibiotics allowed for large improvements to the health care system. Antibiotics, initially penicillin, increased the survival rates of patients by providing a means to counteract infections and they rapidly became a staple in healthcare. While enjoying the benefits of antibiotics, few knew to be concerned about the long-term effects associated with increasing antibiotic use. Even geneticists who specialized in bacteria dismissed any notions of concern by whole-heartedly believing that bacterial mutation rates were too low to allow for resistance to develop [14].

Unfortunately, there were grounds for concern. As antibiotic use persists, infections previously treated with certain antibiotics begin to no longer respond to the same regimens that had once been successful.

An article written by Harold C. Neu in 1992 focused on the appearance of new strains of infections that had become unresponsive to the antibiotic treatments that were in use at that time [60]. At the time of publication, the means by which resistance was transmitted was understood as well as the specific mechanisms that resulted in loss of function for antibiotics. Given this knowledge, the entire article closely explored the increased resistance in specific bacterial infections as well as those observed in broader categories such as enteric, aerobic, and nosocomial infections [60]. Comparing the alterations that have had to be made to treatment strategies over the years is vital to understanding the impact of antibiotic resistance.

Pneumococcal pneumonia and meningitis are infections commonly caused by *Streptococcus pneumoniae*, a gram positive bacterium also associated with infections of the middle ear and sinus cavity [60]. This strain was once easily treated through the administration of penicillin, but failed to respond to that antibiotic by 1992 [60]. A related bacterium, *Streptococcus pyogenes*, is responsible for a slew of infections, including strep throat and impetigo, which were commonly treated with penicillin as well [60]. The use of this specific antibiotic treatment throughout the years eventually saw a time when it no longer produced the same effects on its target. Unlike *S. pneumoniae*, which became entirely resistant to penicillin, *S. pyogenes* only experienced a decreased

response to the antibiotic which was nonetheless detrimental to the success of treating infections caused by this bacterium [60].

When exposed to certain settings or antibiotic treatments, some bacteria are capable of manufacturing enzymes that counteract antibiotics. Unfortunately, this is the case with *Enterococcus faecalis*, which developed the ability to produce penicillinase by 1983 [60]. Penicillinase functions to block the activity of penicillin and thus, is a prime example of the bacteria-born enzymes mentioned above. Globally, *E. faecalis* and other closely related species, cause infections associated with the abdominal cavity, urinary tract, and endocardium of the heart along with other bodily regions [60]. According to Neu, “Enterococci have become the third most commonly encountered, hospital-acquired organism in the United States” [60]. This was a disappointing discovery considering that hospitals see an immense number of patients who may contract an infection caused by these now-penicillin-resistant bacteria [60].

Hospitals are not the only setting hindered by the rise in antibiotic resistance. Across the globe, and especially in developing countries, diarrheal ailments, the result of enteric pathogens, may complicate and take the lives of many. Enteric pathogens are bacteria usually introduced into the human gastrointestinal tract via tainted food. It would be incredibly damaging to those infected if the antibiotics used to counteract the bacteria responsible for these

illnesses were to lose their effectiveness. Unfortunately, that is exactly what happened; *Salmonella*, a member of the family Enterobacteriaceae, and its relative, *Shigella*, cause diarrhea and dysentery, respectively, along with other serious illnesses [60]. In the case of *Salmonella*, routine antibiotic treatments reached a point where they ceased to affect the infection, drastically reducing the number of successful antibiotics [60]. *Vibrio cholera*, another diarrheal disease-causing enteric pathogen infamous as the source of cholera, gradually developed antibiotic resistant strains [60]. Certain antibiotics remain capable of limiting the dissemination of this disease, but many of the more affordable antibiotics, such as tetracycline and sulfonamides, can no longer be used [60]. This particular case not only highlights the increase in antibiotic resistance, but also emphasizes the indirect consequences that a decrease in antibiotic efficacy can produce. Cholera is a disease for which it is crucial to receive treatment, but that help may not always be readily available or affordable. Proper care for this illness has become increasingly challenging since the more affordable antibiotics have lost their effectiveness for this particular bacterium [60].

Numerous examples of common infections, their associated bacteria, and the state of antibiotic treatments for them are described throughout Neu's article [60]. This is a valuable source for developing an understanding of the large-scale effects of antibiotic resistance on the infections that afflict countless individuals.

It may not be difficult to admit that the applicability of antibiotics has changed over the years, but actually learning about the accumulation of bacterial strains involved in antibiotic resistance illuminates the risk it poses. This article documented the transition from a time when nearly all infections could be cured via penicillin to the year 1992, when a shocking number of those infection causing bacteria were no longer thwarted by the tried and true methods. More unsettling is the larger message left in the minds of the reader; if this pattern of increased resistance continues, will the world see a time when we have exhausted antibiotics' usefulness?

Although the previous article thoroughly described the history of antibiotics, it did not delve deeply into the mechanisms behind resistance. However, this topic is addressed by several additional articles, including one by Julian Davies and another by J. L. Martinez and F. Baquero. Additionally, Stuart B. Levy supplied useful information for comprehending how antibiotic resistance occurs. At the time these works were written, it was understood that bacteria multiply via binary fission in which a bacterium is able to replicate itself asexually. Since this form of replication requires only one bacterium, there is no opportunity for genetic recombination and thus the duplicated version is an identical copy of the original. Due to this, if a bacterium has developed antibiotic

resistance and carried out binary fission, the new copy would also possess antibiotic resistance.

A crucial aspect of some bacteria is the presence of plasmids. These circular intracellular structures contain extra DNA that is in addition to the genetic information stored with the bacteria's chromosomes. The origin of plasmids is thought to have been associated with harsh environmental conditions. These challenging conditions were the driving force behind the evolution of plasmids because the genetic information and the genes it corresponded to were not initially suited to allow survival in these settings [37]. However, a bacterium that was capable of surviving in harsh environments most likely had certain traits, encoded by genes, which were advantageous to the bacterium's success. Over time, the accumulation of these types of traits led to the formation of plasmids. There is not one identical plasmid found throughout all bacteria, nor does there have to be only one plasmid per bacterium. Plasmids may not only be unique, there can be a variety of different kinds, allowing for multiple advantageous traits in a single bacterium [37]. Plasmids are beneficial to bacteria because they may impart antibiotic resistance. This process involves existing plasmids merging into larger plasmids while also experiencing losses or additions of genes. These modifications often include the movement of genes coding for antibiotic resistance which may allow bacteria possessing them to

develop resistance [37]. Plasmids that specifically confer antibiotic resistance are often referred as “resistant” or “R” plasmids. Stokes and Hall discovered one way in which plasmids are capable of obtaining new genes is through transposons, specifically, integrons [76]. These are structural components enabling genes to be picked up through a complicated process and eventually incorporated into the organism’s genome [14]. This finding was helpful in understanding how plasmids may obtain antibiotic resistant genes.

It is also commonly known that mutations are the underlying source of new genes and novel traits. Likewise, they play a large role in the development of antibiotic resistance. It has been determined that the presence of antibiotic resistance is dependent on mutations in key genes that are associated with the mechanisms antibiotics employ for success. This includes genes that protect bacteria from foreign invaders such as antibiotics, as well as genes that play a role in accessing, positioning, and synthesizing the antibiotic targets [51]. For antibiotic resistance to develop from mutations, more than one gene may need to be modified via mutation. In some cases, a mutation in a single, key gene could be enough to produce the necessary change, while in others a pathway may incorporate multiple genes which may need to accumulate several mutations. Current methods for examining types of mutations and mutation rates include growing bacterial populations and exposing them to antibiotics *in vitro* as well as

in vivo. Bacteria grown *in vitro* are usually grown in a petri dish or similar piece of laboratory equipment. This allows for reactions between the bacteria and the antibiotic to be observed. In comparison, *in vivo* experiments occur in hosts, usually model organisms, and allow researchers to view the effects on entire individuals. While both methods can offer valuable data, it is important to remember that results of *in vitro* experiments cannot be assumed to also be the same for those *in vivo*. This point was brought up by Martinez, “These differences [*in vivo* vs. *in vitro*] highlight the need for very careful interpretation of the results obtained with current *in vitro* models for the emergence of antibiotic-resistant mutations in bacterial populations” [51]. This is a crucial point to consider when examining the data collected from experiments on antibiotic resistance. It is necessary to not only understand how an antibiotic affects a bacterium, but also the overall impact it will have on the host.

It is important to understand that there is not one universal form of antibiotic resistance. What allows one strain of bacteria to be resistant might be drastically different than the method employed by a different strain. One such mechanism focuses solely on preventing the antibiotic from entering the organism. Antibiotics are designed to repurpose a bacterium’s transport pathways making those pathways likely targets for resistance [37]. If bacteria can block those pathways or somehow render the antibiotic ineffective, then it will

decrease the amount of antibiotic that can enter. Since bacteria typically have multitudes of transport pathways, completely preventing an antibiotic's entry is difficult. Regardless, this method does hinder antibiotics, a point which was noted by Levy, who wrote, "Decreased permeability, however, when accompanying other mechanisms of resistance, can provide the host with very high levels of resistance that are insurmountable by increased antibiotic dosage" [37]. Since there are numerous transport pathways, it is probable that many bacteria have not evolved the ability to thwart all antibiotics, thus increasing the dosage tends to limit the effects of this form of resistance. However, if this method functions in accordance with others, then it could definitely harm the antibiotic's chances for success. [37].

While the method of resistance previously discussed applies to external bacterial defenses, there is a larger variety of mechanisms that can operate in the bacterium. The simplest strategy is to remove the antibiotic from the bacterium before it has had a chance to harm it. Bacteria accomplish this by modifying their cellular machinery to export the antibiotic at a faster rate than it can enter the cell [37]. If the antibiotic is not able to accumulate, it has fewer opportunities to damage the bacterium. At the same time, there are some bacteria that possess the ability to prevent antibiotics from ever accomplishing their tasks. In successfully blocking antibiotics, some bacteria have developed enzymes that

completely protect them from the antibiotic, while others have acquired mutations in the specific cellular component the antibiotic targets [37]. The targets are often structures necessary for the bacteria to survive, eliminating the possibility of simply destroying those components. Instead, a specific mutation may allow that portion of the cell to maintain its function while being altered enough to inhibit the antibiotic from interacting with it [37]. Along with modifying the target structure, some bacteria are capable of producing functionally identical components to replace those that have been rendered unusable by the antibiotic.

A final mechanism of resistance destroys the antibiotic once it is inside the bacterium. This method is associated with a topic previously mentioned and is demonstrated by the ability of *Enterococcus faecalis* to produce penicillinase, antibiotic-blocking enzyme. Penicillinase is not the sole enzyme responsible for blocking antibiotics, but it is a prime example of this mechanism. The antibiotic penetrates the bacterium, but once inside, it will be destroyed or modified by an enzyme prior to inflicting any damage. Several different kinds of this type of enzyme have been discovered in bacteria. Each enzyme is unique in the antibiotic that it targets as well as its mode of action. The ways an antibiotic is blocked can range from destroying the drug altogether to a minute modification that disables the antibiotic thus preventing it from reaching its target [37].

Despite the variety of mechanisms employed, bacteria have developed novel means to resist antibiotics. As described, some mechanisms are more successful than others. Some operate strongly under small amounts of a specific antibiotic, but falter at higher dosages, while others remain operational regardless of the amount. Nevertheless, all these methods are successful to some degree, and are certainly important for understanding the issue of antibiotic resistance in general. Much information regarding gene exchange, the way bacteria can transfer genetic information, had already been compiled prior to the 21st century. For example, it was found that some species of bacteria are able to produce chemical substances known as pheromones. Typically, the bacterium providing the genetic information releases this signal with the purpose of attracting another bacterium [37]. Genetic material is then transferred upon their contact. Additional references describe other forms of gene exchange. These methods include conjugation, transduction, transposition, and transformation and allow bacteria to obtain new genetic information. Collectively, conjugation, transduction, and transformation are forms of horizontal gene transfer. This type of transfer involves the movement of genes from one bacterium to another by means other than reproduction. Although these three methods are all categorized as horizontal gene transfer, the mechanism by which genetic information is transferred is different in each.

Genetic exchange via conjugation occurs by the movement of plasmids from one bacterium to another. Typically, one bacterium contains a plasmid while the partner in the exchange does not. The plasmid-containing bacterium forms a long, thin, structure made of proteins known as a “sex pilus.” This is a relatively complex structure composed of 10-15 specific proteins, produced by genes which are thought to only function in the formation of the pilus [14]. The structure grows toward the other bacterium, drawing the two closer together thus providing a bridge for the plasmid to follow. Prior to the exchange, the first bacterium duplicates the plasmid(s) that will be transferred and then allows the copy to be sent to the other bacterium via the pilus [14]. Upon receipt of the plasmid, both bacteria now possess whatever mutations the plasmid contained and are each able to transfer this plasmid numerous times, significantly increasing the number of bacteria containing those specific mutations. This explains how a bacterial population can quickly accumulate antibiotic resistance, assuming that the exchanged plasmid harbored mutations making resistance possible.

Initially, the process of conjugation was not well understood and was believed to be highly limited compared to the other methods of gene exchange. Although conjugation was documented among a variety of cellular organisms, it was long believed that it could not occur between gram-positive and gram-

negative bacteria [14]. The advent of DNA sequencing techniques aided scientists in documenting that conjugation was frequently employed in nature, even among bacteria [14]. It has also been noted that conjugation is not a species-specific process, meaning that different species of bacteria may engage in conjugation with one another [14]. As the knowledge of this process accumulated, it became clear how conjugation contributes to the spread of antibiotic resistance.

Transduction, unlike conjugation, does not require direct contact between bacteria when exchanging genetic information. Rather than transferring a plasmid from one source to another via a pilus, transduction makes use of a bacteriophage. The vector is a virus specific to bacteria that is capable of storing genetic information. Separate from conjugation, which can only share genetic information residing in plasmids, bacteriophages may contain information from both plasmids and chromosomes. In order for the information to enter a bacterium, the bacteriophage must first arrive at the cells of the bacteria's host. However, the bacteriophage cannot affect just any host cell. It requires the presence of a specific membrane attachment point if it is to infect the host cells along with the DNA it is carrying [37]. Upon receipt of the information by the host cell, the DNA can follow one of two paths. Both of which release this information into the rest of the organism, but vary from one another based on

the time needed for this spread to occur. One option is to immediately eliminate the host cell by rapid multiplication and release of new bacteriophages through the cell's destruction. The second is to remain in the cell, usually attached to an existing chromosome [37]. This option allows both new DNA and preexisting DNA to exit the cell at a later time after the bacteriophage has duplicated itself. The combination of genetic information can then be transferred to more cells as the phage infects them. This movement of genetic material allows for different genes to be exchanged. If the bacteriophage contains a mutation that allows antibiotic resistance, then that ability can be dispersed throughout numerous cells as the phage infects them.

The final form of horizontal gene transfer, transformation, does not require a plasmid or a bacteriophage to accomplish genetic exchange, nor does it need direct contact with another organism. Instead, genetic information is acquired directly from the environment through the cell membrane. This method is challenging to explain because it is difficult to fully characterize the extent of the environment from which the bacteria obtains the genetic material [14]. By 1994, it was accepted that a large amount of transformation occurred among soil microbes and the internal environments of organisms. However, at the time, there was only a limited understanding of how genes were transferred or how many steps were involved for transformation in such environments [14].

Despite this, the importance of transformation was not overlooked by Davies, who stated “Transformation of DNA is likely to be an equally significant resistance gene transfer process in nature, as most bacterial genera can be shown to be competent for transformation under some conditions” [14]. Based on this, transformation appears to be a promising source of genetic transfer and one for which more understanding is needed.

An additional form of genetic exchange is the process of transposition. This method takes advantage of small pieces of DNA known as transposons, and uses them to pass genetic information to another bacterium. Transposons possess the unique ability to move themselves around and insert into various places throughout the genome of the organism they reside in. The movement of these components is often referred to as “jumping.” Their ability to jump increases the survival of the transposons, because it eliminates their dependence on a host cell. Unlike a plasmid, which must be successfully inserted into the host prior to multiplication, a transposon is capable of merely joining with an already established plasmid or preexisting chromosome [37]. Once the transposon has imbedded itself in its new location, it can impart its resistance or whatever mutations it possesses into the host. Since transposons are not constrained by where they can insert, they make reliable vehicles for the transport of resistant genes from one bacterium to another [37].

A considerable amount of information has been collected regarding the mechanisms operating to spread resistant genes between bacteria. It appears that a majority of these mechanisms rely on genetic structures to accomplish the transmission. There are many differences between these structures, as well as their interaction with host cells at the cellular level. Regardless of whether bacteria move genetic information by transposition via transposons, transformation via bacteriophages, conjugation via plasmids, or transduction from the environment, it is apparent that the use of any of these methods could allow for the dissemination of antibiotic resistance. As mentioned for conjugation, there is still much to learn of these processes. A greater understanding may translate into increased success in preventing resistance.

A final commonality among references regarding antibiotic resistance is the use of the antibiotics. Most notably is the use of antibiotics in agriculture. Khachatourians, explained. "In addition to medical misuse, inappropriate use of antibiotics in the agriculture setting is a major contributor to the emergence of antibiotic-resistant bacteria" [32]. Using antibiotics in agriculture has gained popularity over the years. In 1998, this practice saw a range from 100 to 1000-fold increase in the antibiotic use in animals compared to use in humans [32]. Unfortunately, it is not just misuse and increase of antibiotics that is at issue, but

even the methods that are in use to dispense necessary antibiotics may have a role in the spread of resistance as well.

As with humans, plants and other animals are susceptible to microbial infections, and similarly, they may be treatable with antibiotics. However, it is not always as simple to administer antibiotics to an animal or plant as it is with a human, especially if the individual is one of thousands in a herd or planted field. In large-scale operations, it becomes increasingly difficult to limit dosages of antibiotics to a single organism. Due to this, antibiotics aimed at stopping a current infection or preventing anticipated ones are often given to entire groups of animals or fields of plants [37]. Although the consequences of this may seem negligible, it is important to remember that antibiotics often eliminate not only the target bacteria, but may also decimate populations of benign, necessary bacteria such as those of the human intestinal tract [37]. When multiple organisms experience these effects, due to mass exposure to antibiotics, it could provide more opportunities for mutations to appear.

Although a reasonable amount of the antibiotic use in agriculture is appropriately administered for the treatment and / or prevention of infections, there still remains a significant number of misapplications. “About 90% of the antibiotics used in agriculture are given as growth-promoting and prophylactic agents rather than to treat infection” [32]. This suggests that antibiotics could

increase both the weight and proportion of profitable meat when incorporated into livestock feed. Accompanying this finding was a rise in the amount of antibiotics used in agriculture specifically for this purpose. Larger livestock equates to more meat which produces a higher profit for the owner. With this view in mind, incorporating antibiotics into feed became the rule rather than the exception. In 1998, the existing recommended level of antibiotics in feed was 20 times greater than the maximum amount of 5-10 parts per million recommended in the 1950s [32]. This nearly fifty-year period saw a dramatic increase in what was deemed an acceptable amount of antibiotics for animals.

Despite the fact that antibiotic use within the parameters stated above is permissible, it still can have dangerous consequences. Exposing livestock and plants to even minimal levels of antibiotics sets the stage for the selection of resistant genes [32]. This is especially harmful if the levels of antibiotics are too low to eliminate an infection completely. In this situation, bacteria have been exposed to that form of treatment and mutations may have developed. These inadequate levels of antibiotics have conditioned the bacteria, and random mutations, including those conferring resistance, may increase in frequency in bacterial populations. As of 1992, there was no way to even test for resistant mutations among the organisms used by the agricultural industry [37]. The lack of a test meant that the allowable maximums for antibiotic use were being

increased without any knowledge of the number of resistant bacteria in those organisms. The resistance produced in livestock and crops may not only affect the organisms directly exposed, but may could transmit to other animals and plants. The feces from livestock could harbor resistant bacteria while the irrigation run off from crops may contain remnants of the antibiotics administered [32]. If these sources of antibiotics find their way into surface water, they may be spread to other organisms unintentionally. Due to this, neighboring farms may be exposed to the same antibiotics and present the same strains of resistant bacteria even though only one farm uses antibiotics. The use and possible negative results are not limited to specific farms. The fear is that antibiotics and strains of resistant bacteria may end up on produce or in meat products which then provides a direct pathway into humans thus increasing the number of resistant bacteria already present in the general population. There is still much to learn about the impact of a single antibiotic present in agriculture, and the importance of this knowledge increases with increasing antibiotic use.

A considerable amount of information pertaining to bacteria and antibiotic resistance has been collected during the last century. Understanding the mechanisms behind resistance is just as important as realizing the impact of antibiotics. Publications that compare the level of resistance and effective dosage amounts from the beginning of antibiotic usage, compared to the

changes in bacteria observed in the years following, strongly emphasize the shifting world of antibiotics and microbes [60]. This initial information has been supplemented with the elucidation of the cellular mechanisms behind the transmission of resistance. Understanding how mutations in specific genes are often targets for the development of antibiotic resistance established a foundation for expanding our antibiotic efforts. References also describe the forms of genetic exchange available to bacteria along with the strategies employed to diminish and eliminate the effects of antibiotics. Finally, the expanded use of antibiotics in the agricultural industry illustrates the improper use of antibiotics and highlights areas of concern. Although numerous articles written in the same time period are not discussed here, those that have been considered seem to be indicative of the level of understanding of antibiotic resistance prior to 2000. Despite the current wealth of information regarding antibiotic resistance, there remains room for additional information and understanding. As our understanding of this issue increases, so should our ability to respond to and prevent future negative consequences of antibiotic resistance.

III. ANTIBIOTIC RESISTANT INFECTIONS

There was once a time when people could seek the aid of a physician for a simple infection and be confident that their ailment would be cured. The unfortunate truth though, is that those simple infections may no longer be easy

to cure. Despite the impressive breadth of knowledge and array of antibiotics available to doctors, successful treatment may not be possible because those infections have become resistant to the antibiotics that were previously used against them. In reality, antibiotic resistant microbes are a worrisome threat in today's world. A handful of bacteria have developed resistance over the years contributing to infections that are untouchable by antibiotics. Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus epidermidis* (MRSE) are two such examples.

MRSA

A gram-positive bacterium, *Staphylococcus aureus*, was discovered in the 1880s. Prior to its resistance to antibiotics, this bacterium was known for causing infections in post-operative patients as well as small-scale skin infections [16]. Until the introduction of penicillin, *S. aureus* was lethal in 80% of those infections [73]. Penicillin provided an effective weapon to use against *S. aureus*, but unfortunately, not one that would be long-lived. Only two years after the introduction of penicillin into the healthcare world, *S. aureus* developed resistance to it [16]. To combat this development, methicillin, an antibiotic in the same class as penicillin, was introduced. Methicillin was made available in 1959 and had been in use for only two years when a case of *S. aureus* methicillin resistance was reported from the United Kingdom [16,20]. Reports of a similar

nature from across the world followed shortly thereafter. The prevalence of MRSA cases are varied across the globe with some countries being more affected than others. The United States, Japan, South America, and southern European countries typically maintain the highest rates of infection while Switzerland, Scandinavia, and The Netherlands have reportedly the lowest rates [17,58,80].

MRSA infections are usually nosocomial infections, which are secondary infections contracted while in hospitals. Individuals are admitted into the hospital to have a surgery or for an infection not related to MRSA and then, while being treated for their initial ailment, are exposed to MRSA. Sometimes an open wound is not the point of entry for MRSA, but rather it is contact with hospital equipment such as a catheter or respirator [29]. Regardless of the source of the bacterium, MRSA infections have been increasing in hospitals around the globe and, as of the 1990s, have expanded into the general community as well. The two primary locations of occurrence have given rise to unique and distinctly named infections. The methicillin-resistant strains of *S. aureus* found in medical settings are expectedly termed hospital-associated MRSA (HA-MRSA) while the strains residing in communities are community-associated MRSA (CA-MRSA). Although both types of bacteria are resistant to methicillin, they differ on a genetic level.

The methicillin resistance of the *S. aureus* bacterium is conferred from the gene *mecA*, which is only found in resistant strains [7,16]. This gene is responsible

for the production of PBP2a, a penicillin-binding protein that prevents the attachment of beta-lactam antibiotics [7,16,20]. The result of PBP2a is resistance to all beta-lactam antibiotics including methicillin. *MecA* is located in the *S. aureus* chromosome on the staphylococcal cassette chromosome met (SCCmec), a mobile genetic element [7,16,20]. As of 2008, seven distinct types of SCCmec had been discovered [16]. The types differ from one another by molecular weight and can be separated into two groups depending on the resistance they confer. Types I, IV, V, VI, and VII are only capable of producing resistance to beta-lactam antibiotics while types II and III contain extra genes allowing for resistance to multiple antibiotic classes [16]. The varied effects among the SCCmec types contribute to some of the differences observed between HA-MRSA and CA-MRSA. CA-MRSA is resistant to only a small selection of antibiotics and normally contains SCCmec types IV and V, whereas HA-MRSA is usually characterized by resistance to multiple drugs and harbors SCCmec types I, II, and III [21,34].

The variation between HA-MRSA and CA-MRSA does not end with the differences in SCCmec types, but also includes separate demographics of those at risk of an infection as well as unique types of resulting infections. HA-MRSA generally causes respiratory, urinary, and bloodstream infections [34]. In contrast, CA-MRSA results in mild to severe infections of the skin and soft tissue [34]. This includes conditions such as impetigo, cellulitis, and folliculitis as well as

abscesses and necrotizing pneumonia [34]. The infections produced from the community-associated form of *S. aureus* comprise a wider assortment of infections compared to the hospital-associated strain. This is thought to be due, in part, to the presence of supplementary genetic material observed in CA-MRSA. In addition to the SCCmec types IV and V, CA-MRSA contains specific virulence factors known as Panton-Valentine leucocidin (PVL) [21,34]. The cytokines involved in the activation pathway of leukocyte development and tissue necrosis are induced by these virulence factors (i.e., properties of pathogenic bacteria that facilitate infection) thus directing researchers to associate these genes with the types of infections observed in CA-MRSA [34].

The demographics of those at risk of MRSA infections also differ. Prior to the discovery of individual hospital and community-associated MRSA strains, the chances of an individual contracting MRSA was predicted using established risk factors. These risk factors include dialysis, recent surgery or hospitalization, exposure to catheters and/or other medical equipment, and/or time spent in long-term care facilities [34]. If a patient has experienced any of these factors they are at a greater risk of contracting a secondary MRSA infection than those who do not fall into any of the aforementioned categories. One study found that at least one risk factor was present in 47.5% of healthy community individuals with MRSA and 85% in hospitalized MRSA patients [65]. However, these risk

factors are not as useful for predicting infections from HA or CA-MRSA. Those infected with HA-MRSA are commonly older individuals expressing at least one of the risk factors. This is not the case for CA-MRSA which has been seen in younger, healthy individuals without previous exposure to any of the risk factors [24,57].

The earliest reported case of community-associated MRSA came from an Aboriginal tribe in Western Australia in 1993 [16]. Since then, CA-MRSA infections have continued to increase and have even been documented in healthcare facilities. Generally, the countries experiencing higher rates of CA-MRSA are those where it is disseminated from communities into hospitals [16]. With CA-MRSA strains residing in hospitals and other healthcare facilities, there is a greater difficulty in discerning whether patients with MRSA have the hospital-associated or community-associated strain. Identifying the appropriate strain is critical for providing the correct treatment considering that both types of MRSA have different antibiotic resistances. As noted earlier, HA-MRSA is multidrug resistant while CA-MRSA is not. In order to overcome this hurdle, the Centers for Disease Control and Prevention established certain criteria to diagnose CA-MRSA infections. A patient is diagnosed with the community-associated form of MRSA if they present the infection within 48 hours of being admitted to a hospital [16]. Additionally, the patient must also have had no exposure to long-term care facilities or previous hospitalizations/surgeries within the past year [16]. They

cannot have a permanent catheter or had any past MRSA exposure/infection [16]. Association with any of the listed experiences could provide an opportunity for a HA-MRSA infection and would make it difficult to discern if the infection under question was truly from the community-associated strain. Aside from these criteria, a genetic analysis of the SCCmec types could also determine the form of MRSA.

Recognizing the differences between HA-MRSA and CA-MRSA is critical in treating these infections considering that the underlying genetics of each type of microbe may influence how that strain responds to treatment. That being said, the first step to treating any MRSA infection is to determine which antibiotics will be the most efficacious treatment [7]. To accomplish this, a sample of the microbe undergoes susceptibility testing in a laboratory setting where the sample is exposed to a range of antibiotics to see which eradicates it. Unfortunately, obtaining the results takes time as the bacterium must be cultured and then subjected to antibiotics. In most situations, it is not realistic to wait for the results before treating the patient. If one were to postpone treatment the infection could worsen and could threaten that person's life if it was not already at that stage. Consequently, while awaiting the results of the susceptibility test, doctors often prescribe treatments for their patients justifiably based on their prior experience and knowledge. To make the best decision in early treatment, it is

important to take into account what portion of the body is infected, the patient's past antibiotic exposure, the local pattern of resistance, and the patient's health [7]. Once the susceptibility test results are complete, the physician can adjust the treatment to incorporate the findings.

Some infections may require more invasive techniques than antibiotic doses. Infections may develop to the point that the site of infection needs to be cleaned, any fluid build-up needs to be drained, and or surgery is required to remove necrotic tissue or foreign bodies. Additionally, healthcare regulations require that individuals with certain infections, such as MRSA, follow contact precautions while they are in a facility [72]. Contact precautions are devised to increase the safety for both infected individuals and those who come in contact with them. Individuals who have been placed under contact precautions during a previous healthcare facility visit are often placed under that category immediately upon any further visits to that facility, regardless of whether they are suffering from an infection [70]. It is likely that this is due to the fact that someone with MRSA may serve as a carrier for it later in life, even in the absence of symptoms.

Determining which antibiotics to employ against MRSA may be made more difficult by the presence of multidrug resistant strains and variations in resistance patterns. Unfortunately, the antibiotic resistance of MRSA varies among strains

and geographic regions. As mentioned previously, different countries harbor different MRSA infections. Treatments often vary by infection and location which makes it increasingly challenging to develop an overarching treatment strategy. Since most of the CA-MRSA strains have not yet developed multidrug resistance, they can be treated with a larger variety of antibiotics. Aminoglycosides are often used in combination with a beta-lactam to treat skin/soft tissue infections, or with a glycopeptide for endocarditis [7]. Again, these treatments are primarily employed while awaiting the results of antibiotic susceptibility testing. HA-MRSA infections are often times more challenging to treat because they are usually multidrug resistant. In such situations, one of the few remaining treatment options is glycopeptides, particularly vancomycin and teicoplanin [7]. A treatment's effectiveness depends on the site of the MRSA infection. If the infection is in a difficult spot to penetrate, such as an infection in the central nervous system, these antibiotics may be of little use [7]. Additionally, using these antibiotics introduces the risk of the bacterium developing resistance to them. Since glycopeptides are one of our last lines of defense against HA-MRSA, it is critical that they are used appropriately. Patients undergoing glycopeptide treatment need to be monitored to ensure they are receiving the proper dosage in an effort to eliminate the risk of the infection developing resistance [7]. Also, they should only be used when no other antibiotics have been successful. As

with glycopeptides, and for both the safety of the patient and the elimination of the infection, antibiotic treatment regimens need to be carefully followed. This is primarily due to the fact that *S. aureus* infections, like MRSA, often result in complications during later stages of treatment [25].

Once an individual has contracted a MRSA infection, regardless of its origin, it is crucial to treat it. However, it is more important to prevent these infections whenever possible, thus eliminating the need for treatment. First and foremost, in an effort to prevent MRSA, all hospitals, clinics, care facilities, etc., need to ensure that proper regulations are being followed. There are already established measures aimed at preventing the spread of infectious contagions/microbes [59]. Of these procedures, frequent handwashing prior to and after patient contact is important. Some have even suggested that this step is of the utmost importance in preventing the spread of MRSA [66,85]. This may seem a fairly obvious measure, one that most individuals have been reminded of since childhood. It would probably be difficult to find anyone to argue with the benefits of handwashing. Despite the benefit and apparent acceptance of this practice, it is often the main regulation that is not fully observed in healthcare settings [59,67].

Additional precautions are more involved than simply washing hands and following preset procedures. Research has suggested using decolonization

procedures on skin to prevent anyone from carrying the infection topically [47]. Carrying strains of MRSA in this manner not only increases the potential of transmitting the infection to other individuals, but also places that person at greater risk of a relapsing infection. In order to eliminate MRSA colonies, topical regimens should be used [86]. However, this form of prevention is not common and is often viewed as unnecessary. As of 2008, topical decolonization techniques were not required or suggested by established procedures to decrease MRSA infections in the United States [26].

Given our current understanding about MRSA infections, it is clear that there is still much we have to learn. CA-MRSA is still a fairly new type of MRSA infection and, as such, there are not many studies focusing on community members. Rather, the majority of the studies conducted thus far have focused on MRSA infections in hospital settings [34]. Additional community studies have the potential to elucidate the means by which CA-MRSA is disseminated and may help in developing new strategies to counteract and/or avoid such infections. Other improvements can be made in the procedures that are currently employed when treating individuals. Hand hygiene requires stricter monitoring and increased compliance. Also, contact precautions, mentioned earlier, needs to have more clearly defined procedures. Particularly in dealing with when to forego patient contact precautions. In the absence of specific guidelines from the CDC,

there are inconsistencies as to the length of time an individual should follow contact precautions [69]. It would seem that anyone with a previous or current MRSA infection should be forced to observe such precautions as a way to ensure prevention of exposure to other individuals. Although there is some truth to this, forcing a patient to observe these precautions for lengthy periods of time when that individual is not currently infected has the potential to negatively impact them [12,15,18,28,33,53,56,71,74]. Further improvements focus on expanding available treatments through the development of new antibiotics or the refinement of existing ones.

Although, MRSA is a complicated example of an antibiotic resistant microbe, it remains only a small aspect of the much larger issue of antibiotic resistance. That being said, it does provide a valuable example to illustrate just how dangerous and harmful antibiotic resistance can be. MRSA is a complex infection, from its multidrug resistant strains, to its presence in both hospitals and communities; it is a persistent challenge to treat. As our understanding increases, we may find better ways to prevent, diagnose, treat, and even eliminate the threat of MRSA. This is a lofty goal, and one that parallels our hope for the antibiotic issue as a whole.

IV. CAUSES OF ANTIBIOTIC RESISTANCE

The development of antibiotic resistance is an evolutionary phenomenon occurring at the microbial cellular level. As described in the Literature Review, antibiotic resistance typically develops when random mutations happen in the microbe that hinder or inhibit the efficacy of the antimicrobials being used against it. Although these mutations are specifically responsible for antibiotic resistance, additional factors contribute to this process. There is an acknowledged association between the use of antibiotics and the prevalence of antibiotic resistance [62,82]. As such, a majority of these contributing circumstances are associated with how antibiotics are used, both by doctors and patients. These circumstances include both the misuse and overuse of antibiotics in healthcare and personal settings and the use of antibiotics in livestock and agriculture. Indirect causes consist of, but are not limited to, transportation and the use of antimicrobial and antibacterial products outside of the healthcare industry.

MISUSE AND OVERUSE OF ANTIBIOTICS IN HEALTHCARE SETTINGS

Misusing antibiotics in a healthcare setting can occur in a variety of ways. The misuse or overuse may be the result of certain patients influencing doctor's decisions, lack of readily available or efficient laboratory access, and a desire to prevent worsening conditions. It is also important to recognize that healthcare

settings are not limited to hospitals, clinics, and private practices, but also include residential care facilities, particularly for the elderly. Such facilities are often overlooked when considering antibiotic resistance despite the impact they have on the healthcare community [78].

When people seek the expertise of a doctor, they harbor certain expectations; the doctor will provide a diagnosis and will suggest a treatment for their ailment, and, in doing so, will provide them with “peace of mind”. Unfortunately, because of these expectations, patients may not be satisfied with a medical visit if they do not receive the expected diagnosis or anticipated prescription. This is especially prevalent with antibiotics [62]. The interaction between the doctor and the patient, or patient’s family, may affect the doctor’s actions by pressuring them into making prescriptions.

Complicating this issue is that not everyone in the general public is aware that antibiotics are not successful at treating viral infections [46]. In addition, it is difficult for patients to determine whether an infection is viral or bacterial without the assistance of a physician. Thus, some patients may see a doctor for some form of a respiratory tract infection, notoriously caused by a virus, and are displeased when they don’t receive antibiotics. They are often under the impression that antibiotics are the necessary treatment [46,64]. In an effort to obtain those medications, patients have admitted to exaggerating their

symptoms to sway the physician's treatment toward antibiotics [64]. If the patient is compelling enough, they may succeed in convincing the doctor that antibiotics are the best solution. Unfortunately, this misuse of antibiotics likely will not alleviate their symptoms, but will expose both the person as well as the environment and those closest to them to an antibiotic. This may provide an opportunity to promote resistance.

Patients are not only able to sway doctor's judgements by falsely representing their symptoms, but also through the relationship they share with them. For many physicians, maintaining good relationships with their patients is important [62]. Patients need to feel comfortable with their physician and trust their judgement. People don't always understand that a doctor withholds antibiotics because it is not appropriate to prescribe them. Due to this, a patient may begin to distrust their doctor if they don't receive certain prescriptions when the patient deems it appropriate and will seek another doctor who will satisfy their demands. A doctor may be biased by this pressure and feel compelled to prescribe antibiotics even when they are not necessary. This situation is common in pediatrics [52]. No one enjoys watching a child suffer in discomfort or pain, but it is often the influence of parents that can encourage physicians to ignore their better judgement. Prescriptions have the ability to make the patient or parent, in this case, feel justified for bringing their child to a doctor and are comforted by

the thought that progress is being made to treat their child [52]. Regardless of the positive effect the dispensing of antibiotics may have, this tactic may be used even when antibiotics are useless in treating the patient's condition [52]. Just as with the previous example, this misuse of antibiotics to appease patients allows for the possibility of resistance.

Many people may have intentionally or accidentally biased their doctor's diagnosis and prescription due to their actions or information they provide. Doctors admit that self-diagnosis, previous healthcare appointments, arguments for non-medical circumstances, and direct requests all fall into the category of patient pressures that provoke doctors to inappropriately prescribe antibiotics [62]. Generally, it is likely that doctors make this decision with the best of intentions for the health and satisfaction of their patients. Likewise, people want to know that their ailments are being recognized and adequately treated. It seems highly unlikely that doctors prescribe and patients request antibiotics with malicious intents, but there are the consequences of resistance to consider.

There are situations in which antibiotics are inappropriately prescribed not to merely appease the patient, but in an effort to keep them healthy. During an appointment, a patient may present symptoms the doctor would associate with a microbial infection, but either s/he does not have the laboratory equipment or access to it to confirm the diagnosis. Should the doctor not prescribe antibiotics

because s/he can't validate her/his observations with results? Additionally, if the laboratory results do not confirm the presence of a bacterium, does the doctor simply accept the findings of the test? If the results are incorrect or not available and the doctor forgoes treatment, the patient could get worse. That is not always a risk physicians are willing to take. It is not uncommon for a doctor to be uncomfortable with diagnostic results fearing an error was made and treat the patient simply to be safe [62]. These situations are a gamble; perhaps the physician's instincts and observations are correct and prescribing an antibiotic prevents additional health complications. Unfortunately, it is also possible that the laboratory results were correct and an antibiotic will be useless.

The previously described scenario frequently occurs in residential care facilities, particularly those for elderly individuals. Over the course of a year, approximately 50-70% of all residents in these facilities will receive, at minimum, one round of antibiotics [84]. It was also determined that 40% of the given antibiotics were not appropriately prescribed for clinical infections [78]. In other words, the health condition of the patient did not justify the antibiotics they received. This relatively high percentage of antibiotic misuse would seem to encourage negative perceptions of the prescribing doctors, but it is necessary to understand the challenging situation eldercare residential facilities pose. Many of the residents in need of medical care do not have the cognitive ability to provide

accurate medical histories [78]. This prevents doctors from understanding which past antibiotics have been prescribed and the patient's reaction to them. Access to samples for cultures and laboratory settings to justify the use of antibiotics are not always available either, as mentioned earlier [78]. In addition to these existing challenges, one of the aspects that seems most difficult is that a majority of the patients in eldercare facilities, because they are elderly, are at risk of particularly damaging outcomes if infections are left untreated. Discovering and treating infections earlier in elderly individuals could prevent further damage to their health [78]. Also, it is valuable to keep in mind that microbial infections in older individuals do not always present with the typical symptoms seen in younger patients [78]. All of these factors require doctors to base their treatments on what they can observe and their medical instincts, taking into account the consequences if they do prescribe antibiotics to someone that does not need them.

These situations of misuse and overuse do not necessarily indicate that these errors are due to incompetence or lack of concern from doctors. It is actually the opposite; physicians are concerned for their patients' health and happiness. They seem to recognize that maintaining relationships based on trust are critical in assisting patients to the best of their abilities and that inappropriate prescriptions may help to preserve that relationship and provide "peace of mind"

at the expense of the best medical solution. In my opinion, based on the situations described above, doctors misuse antibiotics because they fear damaging their patients' health. Despite these best intentions, this misuse and overuse of antibiotics could contribute to antibiotic resistance.

MISUSE AND OVERUSE OF ANTIBIOTICS IN PERSONAL SETTINGS

Inappropriate antibiotic use may not be limited to healthcare facilities nor restricted to the actions of physicians. It likely also extends into personal settings, as well. Noncompliance and self-medication are two possible sources of antibiotic misuse and overuse. The distribution and availability of antibiotics could also contribute to their misuse.

Simply because a physician prescribed a medication with specific instructions does not necessarily mean the person receiving the prescription will follow those directions. Doctors must give their patients the benefit of the doubt when prescribing antibiotics, especially because it is unlikely that self-reporting on noncompliance is completely accurate [64]. Noncompliance does not mean that the patient isn't taking any of their prescribed medication, but actually consists of any behavior that deviates from following the prescribed dosage and directions. This includes failing to complete the entire prescription, altering dosage amounts or frequency of doses, and storing leftover antibiotics for later use.

One study examining patient's understanding and practices with antibiotics found that all antibiotic doses were completed by only three out of four patients [64]. This same study also questioned the reasoning behind prematurely stopping the prescribed antibiotics and found that a majority reported that they felt well enough to not need them [64]. Stopping antibiotic use because the symptoms have disappeared seems to be fairly common, but it is crucial that entire doses are completed. Not complying with the duration of a prescribed antibiotic may result in a reoccurrence of the infection and may unknowingly expose others to the microbe. Additionally, some individuals may alter their daily dosages so that they receive smaller quantities of the drug than prescribed. This action has the potential to promote resistance because the microbe is exposed to nonlethal levels of antibiotic [13].

Self-medicating may also contribute to antibiotic resistance [36]. Individuals may treat themselves with antibiotics that are not appropriate for their ailment. They may administer an antibiotic for a self-limiting condition or one unresponsive to such medications. There is also a risk they will not select the correct antibiotic nor administer the correct dosage or duration. There appears to be numerous unknowns and risks associated with self-medicating. Unfortunately, these actions may expose microbes to incorrect antibiotics or nonlethal doses providing an opportunity for resistance to develop.

The fact that people self-medicate is not the only concern. What is also worrisome is the source of antibiotics. Some individuals will fail to complete their prescribed antibiotics, as mentioned, and will save the remaining doses for future use [64]. In some countries outside of the United States, people do not need to hoard their antibiotics because they are available without prescriptions. One study found that antibiotics were available without prescription in the United Kingdom, Colombia, France, Morocco, Thailand, Turkey, Spain, Italy, and Belgium [64]. It is not that antibiotic sales were legal without a prescription in all these countries, just that it was possible to obtain antibiotics under certain circumstances [64]. People within the nine previously mentioned countries were able to acquire antibiotics from their pharmacist without needing a prescription from a doctor [64]. This dispensing of antibiotics not only contributes to antibiotic resistance by allowing for inappropriate usage, but also encourages self-medicating behavior.

ANTIBIOTIC USE IN LIVESTOCK

When the phrase “antibiotic resistance” is encountered, it is likely that most people immediately think of hospitals, doctors’ offices, and/or people suffering from microbial infections. These associations are certainly appropriate, but do not complete the entire picture of antibiotic resistance. Antibiotics are not solely used in human settings, but are also employed in livestock. It is not

uncommon for animals to receive antimicrobial agents for the prevention, treatment, and control of infections [3,50]. Reasons for their use are similar to why humans receive antibiotics. Also, the conditions of some farms may be enough to warrant the use of antibiotics. Overcrowding and unhygienic conditions, especially in aquaculture [50], arguably makes the use of antibiotics necessary for the health of the animals.

However, during the 1950s, the use of antimicrobial agents began in livestock (poultry, turkey, swine, and beef cattle) to promote growth and contribute to feed efficiency [3,50]. Since livestock are sold as food animals, and larger animals fetch higher revenues, it is not difficult to understand why antimicrobial agents are used. However, unlike taking an antibiotic for an infection, the use of them in livestock is not necessarily for promoting health. Some antimicrobial agents used as growth promoters are given to numerous animals in low doses on a continuous basis for lengthy periods of time. These conditions are ideal for the selection of resistant bacteria [50]. In the United States, as of 2003, 17 classes of antimicrobial agents were considered acceptable for the enhancement of growth and feed efficiency [3]. The approval of antimicrobial use in livestock does not necessarily mean that there are no consequences for this action. In reality, the misuse of antibiotics in agriculture is a contributing factor in antibiotic resistance [32,52].

There are two main concerns about the use of antimicrobial agents in livestock and antibiotic resistance: 1) selection for antibiotic resistant microbes in the livestock which may ultimately harm humans, and 2) it unnecessarily exposes the environment to antibiotics. As in humans, if livestock are exposed to nontherapeutic levels of antibiotics or inappropriate antibiotics for microbial infections, it provides a setting for the selection of microbial resistance. This is enhanced by the fact that most antimicrobial agents are given to livestock in small doses over extended periods of time [50]. An associated concern is whether those antibiotic resistant microbes from livestock have the ability to harm humans. It is possible that resistant strains of bacteria from animals may be antimicrobially resistant in humans if the antimicrobial agents given to livestock are the same or similar to those used in humans [3]. Based on this finding, if an animal harbors a resistant bacterium it may be possible for that bacterium to find its way to humans. Additional evidence was found supporting the existence of both resistant bacteria and associated genes in animal products regardless of the stage of processing for those products [50]. It may not be surprising that meat fresh from the slaughterhouse contains the same bacteria that inhabited the organism that had recently been living. However, the idea that those bacteria are still present after processing may be worrisome.

The products from food animals are not the only source of concern for spreading antibiotic resistance. Direct contact with livestock may also serve as a route for the dissemination of antibiotic resistance. There is a risk to those who work in a close environment with livestock, such as farmers, veterinarians, and/or employees of processing facilities. These individuals may contract antibiotic resistant infections from infected livestock [50]. If those individuals become infected they may pass it to other people with whom they come in contact. Thus, the use of antimicrobial agents in livestock may select for resistant microbial strains that, through both direct and indirect contact, could harm the human population. One study supported the connection between the use of antimicrobial agents and resistant bacteria in both livestock and farmers through a comparison of a farm that promoted growth via antimicrobial agents versus one that did not [50]. Researchers found that both the livestock and the people in contact with the animals receiving antimicrobial agents had resistant strains among their normal intestinal bacteria while the farm that did not use antimicrobial agents did not [50]. This suggests that promoting growth and feed efficiency through antimicrobial agents may also promote resistant bacteria that are not solely restricted to the livestock.

The second concern is that the use of antibiotics in livestock will unintentionally expose the environment to antibiotics. It has been noted that

extended periods of antibiotic use may impact the environment in which the antibiotic is being dispensed [44]. Contributing to this issue is the fact that not all antibiotics leave the body in an inactive form. Unless a resistant bacterial strain is capable of producing enzymes to deactivate an antibiotic, the bacterium may simply change the antibiotic target or remove it from the cell. These latter actions do not do anything to hinder the activity of the antibiotic. Thus, it is possible that active antibiotics enter the environment through the excrement of livestock [44]. Once in the environment, those antibiotics, now at nontherapeutic doses, may end up in food crops or water supplies where they can select for resistant microbes [44,52]. This is a concern not just about the use of antimicrobial agents in promoting growth, but also about dispensing antibiotics in animals in general, as is seen in the aquaculture industry. Providing antibiotics to aquatic organisms, especially fish, can be difficult. To overcome this challenge, the drugs are often combined with food which is then placed in the water [50]. Unfortunately, this practice exposes entire aquaculture systems to antibiotics and if any of that water is released into larger aquatic ecosystems, it could act on pre-existing bacterial strains and may select for resistance among those individuals [50].

Antimicrobial use in the livestock industry is a controversial topic. Animals suffering from bacterial infections obviously cannot be left untreated, despite the fact that the use of those drugs may impact both the human population and the

environment. Similarly, the use of antimicrobial agents as growth promoters may seem an unnecessary risk. However, larger food animals, or animals that produce more products, are preferred over smaller individuals which contributes to greater profits. Regardless of the various motivations for the use of antibiotics in livestock, it may contribute to antibiotic resistance among certain microbes.

ANTBIOTIC USE IN AGRICULTURE

The use of antibiotics in agriculture and livestock share many commonalities. Produce and fruits may come in contact with antibiotics as an indirect result of antibiotic use in livestock. As described previously, the excrement of animals may contain active antibiotics that could reach agricultural products through water run-off or the use of livestock manure as fertilizer. However, produce and fruits are also directly exposed to antibiotics, primarily through the use of pesticides or in treatment of agricultural diseases. Streptomycin™, Gentamicin™, oxytetracycline, and oxolinic acid are commonly used in agricultural settings [75]. In 2002, approximately 45×10^3 kg of antibiotics were used as pesticides solely for fruit trees [44]. There is concern that these antibiotics, just as with livestock, could have the ability to select for resistance in bacterial strains in the surrounding environment.

Thus, there is concern that increases in resistance of bacteria observed in a clinical setting may result from the impact of agricultural antibiotics on the

environment [75]. However, antibiotic residue does not appear to remain active for extended periods of time when used in agriculture [75]. This would suggest that although the antibiotic might be present in the environment, it is not capable of hindering bacteria and wouldn't provide an opportunity to select for resistant microbes. Findings such as the aforementioned, combined with regulated practices (the topic of section V), may minimize the fear that antibiotic use in agriculture enhances antibiotic resistance.

ADDITIONAL CAUSES

Many modes of modern day transportation, ranging from buses to airplanes, seem to make the most efficient use of space. The result is that people are often crowded together and may come in direct contact with each other. If any of those individuals was harboring an infection, for example an antibiotic resistant infection, it would not be difficult to transmit it to others given these crowded conditions [52]. This form of dissemination is not limited to human travel, but is also a factor in livestock transportation as well. Livestock are frequently transported in more crowded conditions than are humans. It is possible that bacterial strains may spread among livestock in that environment as well. Additionally, human patients that are known to be suffering from an illness or infection could likely be transported to or from hospitals and care facilities on occasion, to receive treatment elsewhere. This movement of infected individuals

may increase the number of people they come in contact with, increasing the chances for dissemination to others. Although transportation does not directly select for the resistance of a bacterial strain as does an antibiotic, it does enhance the dissemination of strains that are already resistant. Thus, by providing environments conducive to the spread of contagions, transportation indirectly contributes to the issue of antibiotic resistance.

Another possible cause of antibiotic resistance is the use of antibacterial household products. These products' impact on antibiotic resistance may be less easy to document than the impact of transportation. The number of antibacterial products has increased from only 23 individual items in 1993 to more than 700 in 2002 [44]. These products range from common surface disinfectants to antibacterial soaps and even plastic products infused with antibacterial properties. Regardless of the antibacterial product, the aim is to prevent the spread of contagions and hinder the growth of bacteria [41]. In this way, these products share similarities with antibiotics, but are not the same in terms of modes of action on microbes. However, just as with antibiotic use, there exists concern that the use of antibacterial products may influence antibiotic resistance in microbes to these products.

One study found that the increasing use of these products in residential settings may impact antibiotic resistance [41]. Antibacterial products commonly

contain quaternary ammonium compounds and triclosan [44], the latter of which is of concern for antibiotic resistance. There is some debate over whether antibacterial products actually promote bacterial resistance to triclosan.

Generally, antibacterial soaps contain high enough concentrations of triclosan to eliminate bacteria, but it was found that the combination of triclosan and soap in these products may actually reduce the efficacy of the chemical [41].

Additionally, the conditions (temperature, duration of exposure, and concentration of chemical) under which triclosan must be exposed to the bacterium are rarely achieved with typical hand washing [41].

In contrast, a separate study examined the effects of using antibacterial products, for a one-year period, on antimicrobial resistant organisms residing on hands [1]. The findings did not suggest that the use of these products, during the allotted time span, had a significant effect on increasing antimicrobial resistance [80]. These findings do not necessarily address all concerns about links between antibacterial products and increasing antibiotic resistance. As the use of antibacterial products increases, the potential for resistance to develop to those products may also increase. This is especially possible if the product is capable of leaving a residue, as is seen in antibacterial soaps, but not in peroxides or alcohols [41]. Soap residues may contain low levels of the antibacterial agents which could select for resistant bacteria since these agents may not be potent

enough to eradicate any bacterium that encounters them. There appears to be a general acceptance in the medical community that extended and heightened use of antibacterial products may increase antibiotic resistance among microbes by providing an environment in which resistance is promoted [1,41]. Some researchers support the use of antibacterial products only for protecting recovering patients [41]. Despite this recommendation, antibacterial products seem to have become commonplace in the home.

Both transportation and antibacterial products may not be the primary or most significant causes of antibiotic resistance. This is because their roles as contributors to the problem are not always direct nor completely understood. Nevertheless, it is important that we attempt to understand their impacts, and which areas warrant further investigation.

V. PREVENTING ANTIBIOTIC RESISTANCE: CURRENT PRACTICES

As previously described, antibiotics are used for various treatments in numerous settings. Although antimicrobial compounds have improved healthcare and saved countless lives, their efficacy is limited by the development of antibiotic resistance to them. The existence of antibiotic resistance does not mean that antibiotic use is unregulated. Instead, policies exist and practices are followed in an effort to provide efficient patient care and prevent further antibiotic resistance. These approaches can be grouped into two primary

categories; prescribing practices at the physician level and regulations surrounding the use of antibiotics in agriculture and for livestock. Additional approaches are also employed to diminish or prevent the onset of antibiotic resistance.

PHYSICIAN PRESCRIBING PRACTICES

As previously noted, antibiotic resistance develops, at least in part, to the overuse/misuse of antibiotics prescribing physicians. However, some medical professionals also maintain practices aimed at limiting the opportunities for resistance. In response to antibiotic resistance, physicians in some countries have increased their use of clinical tests in determining the proper course of treatment [52]. By using more clinical tests, those doctors have a better understanding of which microbe plagues their patients and whether that pathogen will respond to antibiotics, and if so, which ones. This approach allows doctors to make informed decisions when prescribing antibiotics which may decrease the opportunities for microbes to be exposed to inappropriate treatments, thereby reducing the possibility of developing resistance.

Also, in an effort to make the most efficient choices, physicians may rely on their medical training and consult prescribing guidelines, decisions trees, or follow care paths [52]. Decision trees incorporate choices and associated aspects that may include consequences or costs of said choice. In the medical field,

decisions trees often contain preferred drug options and recommended durations for patient treatment [52]. Where decision trees may be vague or open for interpretation, care paths can be more specific. A care path, or clinical practice pathway, provides more specific recommendations that follow timelines and are evidence-based [52]. Although some may find a care path more applicable than a decision tree, the use of either is made in an effort to choose the best form of treatment for patients. Either method has the potential to help guide a physician to a treatment that may have a reduced risk of developing antibiotic resistance.

Physicians' treatment decisions may also be guided by computerized antimicrobial approval systems (cAAS) [5]. The Royal Melbourne Hospital, in Australia, monitored drug usage and provided prescribing recommendations through such a system [5]. This cAAS incited prescribers to critically evaluate their medication choices (i.e. the spectrum of the drug used) and treatment durations. By encouraging this type of behavior, prescribers may reconsider their treatment strategies and promote prescriptions that have a reduced likelihood of promoting resistance. A study analyzing the cAAS in the Royal Melbourne hospital found that a variety of restricted drugs had a decreased rate of use compared with previous year's data [5]. The overall conclusion was that the computerized stewardship system was a successful approach for altering antibiotic

consumption in that hospital. It is suggested that such programs could improve patient outcomes and lessen the rate of antibiotic resistance [5].

In conjunction with making informed and legitimate treatment choices, physicians also attempt to avoid antibiotic resistance through their selection of antibiotics. Some doctors will opt to use newer treatments over default approaches in an effort to avoid those drugs bacteria may already have an increased exposure or resistance to [88]. When certain antibiotics are effective at treating bacterial infections, they could easily become the “go-to” drug of choice even if other effective drugs exist. Although those antibiotics may be the best choice, the frequent use of them leads to increased exposure of bacteria to those drugs, which could create an environment encouraging resistance. By using less common treatments, doctors can avoid drugs that already have resistance developed to them. In addition to using new, less common drug compounds, physicians may also attempt to use narrow-spectrum antibiotics whenever possible [19,88]. Although broad-spectrum antibiotics may successfully treat bacterial infections, it may be at the risk of removing the natural flora of the body which in turn could allow additional harmful bacterial to colonize in their place. Narrow-spectrum antibiotics are more specific and limited in what they are effective against [19] and have a reduced likelihood of creating resistance [88]. Doctors may also prescribe multiple drugs rather than a single antibiotic to treat

a bacterial infection [52]. Drug combinations could result in synergistic effects where the drugs involved become more effective. These practices in selecting antibiotics may not always be an option as it depends on the individual and the risk those choices pose to their health. However, by making more informed choices, doctors have the ability to limit the opportunities for resistance to develop.

A final approach doctors employ is not specifically in the antibiotics they prescribe, but in how they prescribe them. As described previously, the misuse of antibiotics results, in part, from patients or relatives of patients expecting antibiotics for ailments that are not amenable to these treatments. Not prescribing antibiotics when a patient expects them may leave a patient feeling dissatisfied with their treatment which could result in loss of trust in their physician. This may place doctors in a difficult predicament; do they inappropriately treat the patient at the risk of contributing to antibiotic resistance in order to maintain the physician-patient relationship, or do they prioritize minimizing antibiotic resistance above their patient's satisfaction? A compromise to this predicament is to delay antibiotic prescriptions. If a doctor is aware that a patient's condition will not improve with antibiotics, they may advise the patient to wait a certain length of time for their ailment to improve [4,52]. If there is no improvement after that time, then the doctor will prescribe

the antibiotic. Infections, such as those of the upper respiratory tract that are caused by viruses and are thought to need antibiotic treatment, but actually don't, will usually improve [4]. Doctors may also opt to write a patient a prescription that cannot be filled until a specific date, a date outside of the time frame [4]. Having such a prescription in their possession, rather than a verbal agreement, may lead to greater patient satisfaction. This approach is often particularly successful when treating children because parents feel that their child's ailment has been acknowledged and efforts have been made to treat it [52]. Whether the delayed prescription is suggested verbally or guaranteed through writing, this can be an effective approach to limiting the inappropriate use of antibiotics.

Not all doctors follow these practices nor are all practices the best options for every patient. These approaches may vary depending on the situation, the resources available, and/or the specific needs of each patient. Despite these limitations, the prescribing practices of doctors have the potential to diminish antibiotic resistance.

REGULATIONS OF ANTIBIOTICS IN AGRICULTURE/LIVESTOCK

Monitoring the prescribing practices of physicians is not the only area in which attempts are made to curb antibiotic resistance. With the application of antibiotics to livestock and agriculture potentially contributing to the

development of resistance in bacterial species, there are regulatory practices and restrictions to prevent and/or lessen the consequences. Some of these approaches attempt to restrict the specific types of antibiotics used, while others aim to minimize human exposure.

Globally, some countries and health organizations have advocated for the complete elimination of the use of any antibiotic compounds for growth promotion that are also used in the treatment of humans [3, 6]. The exception to this recommendation would be if the use of such drugs were completely necessary and unavoidable. Restricting the range of growth promoting antibiotics to those that do not have a human analogue could decrease the selection of resistance to those antibiotics and maintain their effectiveness in humans. Although this could be a beneficial restriction, not all countries comply with this suggested course of action [3]. However, some countries in the European Union have completely banned the use of growth promoters in food animals, horticulture, and aquaculture to prevent the increase of antibiotic resistance [2].

Aside from regulating which antibiotics are recommended or legally permissible for use in agriculture, there are guidelines to reduce or prevent human exposure. These regulations establish that individuals distributing antibiotics must wear personal protective gear to prevent direct contact with the antibiotics [75]. Additionally, humans have to wait specific periods of time before

entering an area that has been treated by antibiotics [75]. Similarly, there are certain intervals that must occur between applying antibiotics and harvesting crops [75]. These practices aim to prevent humans from encountering any lingering antibiotics, thus reducing the likelihood that they would be exposed to nontherapeutic levels that could select for resistance in infectious bacteria.

In an effort to reduce direct and indirect human contact with antibiotics, the conditions that livestock are raised in and how these conditions contribute to antibiotic resistance needs to be considered. Use of vaccines, accompanied by heightened sanitary conditions in livestock operations, particularly the aquaculture industry, reduces the possibility of infections and ailments in the animals [6]. Reducing the requirement for antibiotics, in turn, limits the direct exposure of antibiotics to animals and the dissemination of those antibiotic residues into the environment [6].

ADDITIONAL PREVENTATIVE MEASURES

Aside from practices in the healthcare, agriculture, and livestock industries, there are broad, daily approaches that may prevent the dissemination of resistance. Additional efforts include promoting patient education, maintaining infection control measures, and encouraging vaccination and proper hygiene. Providing multiple sources of information and education can help patients be better informed about antibiotic resistance and appropriate antibiotic practices

[46]. For example, if patients can understand that their viral infections will not improve with antibiotic use, this understanding can diminish the pressure they put on their physicians for inappropriate prescriptions. Regulating infection control practices, vaccinations, and hygiene practices can prevent the transmission of infectious microbes [1,52]. Decreasing the dissemination of infectious microbes reduces the morbidity of associated infections resulting in a decreased need for, and distribution of, antibiotics. Since a reduction in antibiotic use is associated with less resistance, these practices have the potential to prevent or decrease antibiotic resistance [46].

Whether its physician practice and the guidance they receive, regulations, or education of the general public, there are numerous efforts underway to halt the increase of antibiotic resistance. Regardless of their success, all efforts must be evaluated on a case-by-case basis. Although these efforts are critical in reducing the impact and incidence of antibiotic resistance, there remains room for improvement.

VI. PREVENTING ANTIBIOTIC RESISTANCE: RECOMMENDATIONS FOR FUTURE PRACTICES

Antibiotic resistance has become a public health concern with no simple or immediate solution. Current regulations followed and approaches practiced by physicians, the general public, and those in the agricultural/livestock industry are

valuable in diminishing the selection of antibiotic resistant microbes. However, these methods alone are not enough to prevent our “miracle drugs” from becoming ineffective. To sustain a future in which antibiotics can are still efficacious, we must maintain our current efforts as well as implement new strategies in the following areas: physician education and practices, patient education and practices, regulations regarding the use of antibiotics, and drug development.

PHYSICIAN EDUCATION

Within the healthcare industry, the pervasiveness of antibiotic resistance is well known. Despite understanding the issue, numerous physicians view antibiotic resistance as a concern for secondary healthcare facilities rather than their primary practices [82,88]. Some physicians may fail to make the connection between their prescribed antibiotics and diminished effectiveness because of microbial resistance to the drug. For example, if a patient responds poorly to an antibiotic treatment, it is thought to be due to either the presence of a viral pathogen or noncompliance with the physician’s instructions [88]. Others harbor the belief that those at fault for resistance are the dentists, veterinarians, pharmaceutical companies, and pharmacists who inappropriately prescribe and/or use antibiotics [52]. Additionally, there are those who maintain the view that antibiotic resistance is a significant issue, but do not believe that their

actions alone will “fix” the problem [52]. Thus, these physicians do not want to risk the health of their patients through limiting their prescriptions since their actions will not have a noticeable impact on antibiotic resistance. Although this is certainly not the perspective harbored by all physicians, it is likely that there are some who maintain these views.

If the future of antibiotic resistance is to improve, physicians need to abandon the notion that antibiotic resistance is restricted to secondary care. One strategy to address this false notion is to provide local resistance data to primary care practices [88]. This information would allow physicians to be more up-to-date in their knowledge of which bacterial infections are currently showing resistance in their practice area. This knowledge could alter and improve their treatments. It could also be used to verify resistance as the problem when patients don’t respond as anticipated. Both patient health and prescribing practices have the potential to improve through the realization that antibiotic resistance is capable of being an active agent in primary care, as well as secondary care, facilities.

It is also crucial to abandon the practice of blaming others for the problem [82]. Numerous contributors, ranging from doctors to patients to pharmaceutical companies may play a role in encouraging the resistance problem, but no one individual is solely responsible. Overcoming the desire to place blame elsewhere

will allow more focus on the issue itself. This will encourage all parties to make their best efforts to avoid exacerbating the problem further. The view that one doctor's actions will only negligibly alter resistance may be accurate, but if each person, whether doctor, patient, or pharmacist, were aware of the issue and made every effort not to contribute to it, then the culmination of all those efforts could alter the future of antibiotic resistance for the better.

PHYSICIAN PRACTICES

A physician's prescribing practices may be influenced by their education, their personal experiences or the experiences of their mentors, and/or their patients. As previously described, providing more resistance data and understanding the role physicians play in antibiotic resistance is crucial to changing the course of resistance, but it is not the only physician-related aspect that should be changed. There is also room of improvement in the prescribing practices of physicians.

At the forefront of proper prescribing is the ability to correctly identify which microbes are infecting patients. Correct diagnosis does not only stem from a medical education, but also from laboratory testing. One study found that the availability of laboratory data impacted physician's knowledge of antibiotic resistance [88]. An increased availability and use of point-of-care tests, such as the Strep A test or CRP rapid testing, can eliminate diagnostic uncertainty [46,52,

62]. If doctors know for certain that a patient's condition will improve with antibiotics, then they can make appropriate prescriptions that will have a reduced chance of selecting for resistance in that bacterial species. Many facilities already implement laboratory tests such as these, but not all physicians have access to the necessary equipment [46]. If the equipment is not in-house, then the results could take longer to arrive since they must be outsourced. Doctors may feel obligated to opt for prescriptions without the confirmation of diagnostic results if there is a chance the patients' condition may worsen during the time it takes to identify the specific microbe. This concern is also associated with some diagnostic tests that have lengthy waiting periods before results are available. For example, to state that a certain patient's culture has "no growth" takes at least two days using common culturing methods [46]. Waiting two days before providing a patient with treatment may be detrimental to their health and/or the doctor-patient relationship. Thus, while the increased use of diagnostic tests can guide physicians in issuing appropriate prescriptions, an improvement in testing techniques may also improve prescribing practices and reduce the selection for resistance.

Aside from identifying the microbe of interest to ensure appropriate treatment, there are additional ways in which physicians can improve their practices. Some argue that prescribing fewer antibiotics will decrease the

chances of contributing to resistance [88]. If fewer antibiotics are in circulation, then fewer bacteria will be exposed to them diminishing selective pressure. It may be possible to reduce prescribed antibiotics by promoting practical antibiotic practices [46]. In other words, antibiotics should be prescribed only when the doctor is certain the patient will receive significant benefit from their use.

Adopting this approach would mean fewer prescriptions for viral infections.

Opting for courses of treatment shorter than 10-14days can also reduce microbial exposure to antibiotics [44,52]. Some would argue that shorter courses may not completely eradicate a bacterial infection, but there have been few studies to suggest the benefit of using 10-14 day regimens [52]. Additionally, cycling antibiotics, or rotating which ones are frequently used, may prevent further resistance development by limiting the exposure of microbes to those drugs [44]. Despite the hypothetical benefit associated with the practice of cycling, there is little evidence to actually support it [52].

Implementing one or more of these prescribing practices as a determinant in the physicians' pay may encourage more progressive behavior and encourage accurate prescribing practices. This strategy could monetarily reward doctors for limited use and accuracy of antibiotic prescriptions [52]. However, this may or may not be a successful approach in motivating physicians to curb their antibiotic prescribing. The culmination of these efforts, whether it be an increase in

diagnostic testing, prescribing fewer antibiotics, and/or providing monetary motivation for appropriate prescribing, are ways we can hopefully prevent future antibiotic resistance.

PATIENT EDUCATION

In a world with antibiotic resistant microbes, having well-educated patients is paramount. As described in an earlier section, patients can exert tremendous pressure on a physician's prescribing practices. If patients have a better understanding of the appropriate uses for antibiotics, they may put less pressure on their physicians to inappropriately supply them with those drugs. This will not only diminish the misuse and overuse of antibiotics in healthcare settings, it could also encourage proper use in personal settings. Promoting these practices could eliminate some of the opportunities for resistance.

Patient education can occur through many avenues, including informational pamphlets, media coverage, K-12 classroom lessons, and conversations with medical professionals [32,52,62,64]. Although the issue of antibiotic resistance is widely known in the healthcare community, the same may not be true for the general public. It is important to ensure that patients are not only aware of antibiotic resistance, but that they also understand when antibiotics should be used (e.g., for bacterial infections, but not for viral), the consequences of misusing antibiotics, and why it is critical to comply with drug

instructions [52,64]. The doctor-patient relationship allows doctors to play a vital role in educating their patients [62]. Physicians are not limited to simply informing their patients of the dangers of inappropriate antibiotic use; they can also help patients feel more comfortable regardless of the recommended course of treatment. Physicians can discuss what the patient should expect from their treatment, how long symptoms should linger, and when it is appropriate to contact the physician again [46]. By ensuring that patients understand and feel comfortable with this information, they may have more trust in their doctor's recommended treatments even if those regimens do not include prescriptions for antibiotics. Educating and conversing with patients could be one way to reduce the misuse of antibiotics, potentially without sacrificing the satisfaction and trust of the patient.

Many doctors are limited to only a few minutes of consultation with their patients. This time allotment makes it difficult to adequately explain their prescription choices and to discuss any concerns with their patients [62]. One way to overcome this difficulty is to combine a physician's verbal advice with printed material [62]. This approach provides the physician with time to explain their concerns while giving the patient additional information they can consult at a later time.

Educating patients via one of these methods alone may not reverse the misunderstanding that antibiotics are appropriate for all ailments [46]. Instead, providing multiple sources of information through various means may help patients become well-informed and increase the appropriate use of antibiotics. It is also important to implement educational methods that are tailored to specific regions [62]. Just as doctors have argued that having local resistance data would be helpful, so too would be patient education that addressed the specific concerns for the areas in which the patients reside [62]. Antibiotics and the associated concerns for their use are perceived differently depending on the cultural lens they are viewed through. Thus, an educational approach that might be successful in one country may not engage the target audience of another. Through a multi-pronged, country-specific approach, patients can become more educated about antibiotics, the issue of resistance, and the role they play.

PATIENT PRACTICES

There are multiple ways in which the practices of patients and the general public can diminish, or at least not increase, antibiotic resistance. Complying with treatment directions will ensure that bacterial infections are treated with therapeutic levels and for long enough durations to eliminate infections. Not adhering to those guidelines may destroy only a portion of the bacterial

population, while leaving the remaining members alive and exposed to that antibiotic. This creates situations ideal for selecting for resistance.

Another obvious and crucial strategy, at both the patient and physician levels, is to prevent the transmission of resistant strains. An excellent way to pursue this approach is through proper hand hygiene [32,61]. Although developed nations maintain and promote such practices, developing nations may not have the resources or programs to prevent infections that spread this way [61]. Also, taking advantage of vaccinations and making vaccinations globally available could contribute to reducing antibiotic resistance among microbes [52]. If all humans adopted these practices, antibiotic resistance could be reduced and possibly prevented.

ANTIBIOTIC GUIDELINES

As previously explained, strategies focusing on the education and antibiotic habits of patients and physicians appear to be valuable in reducing the presence of antibiotic resistance. Nevertheless, additional antibiotic regulations could also be beneficial. Specifically, improvement in drug guidelines will help to maintain the efficacy of current antibiotics and diminish their misuse [52,62].

Some studies have suggested a connection between inappropriate prescribing practices and poorly constructed antibiotic guidelines [52,62]. Guidelines exist to advise prescribers in their treatment choices. Although a

physician's judgement may be positively influenced by guidelines, often the guidelines can only provide suggestions, leaving the ultimate decision up to the physician [52]. In this way, the consequent actions of a physician may not have been affected by the guidelines whatsoever. Some guidelines are too focused on selecting the correct drug and neglect to provide information suggesting shorter durations or the option to withhold treatment altogether [52]. Selecting the appropriate drugs are indispensable in providing proper treatment, however, as previously described, not all situations may call for antibiotics. Guidelines that present the drug choice as the primary concern over the decision to treat may mislead prescribers. Additionally, pharmaceutical companies may frequently promote specific guidelines if one of their products are included in them [52]. The support of a pharmaceutical company may create the appearance that their drug is the premier option, thus encouraging physicians to employ that drug over others that are equally effective.

Based on these drawbacks, the creation of new guidelines could encourage more progressive prescribing. Guidelines could include pathways (e.g., decision trees) to help physicians decide if antibiotic treatment for a patient is appropriate and, if so, suggestions for limited durations to avoid prolonged exposure. Some studies also suggest that guidelines should be created by multiple medical disciplines to encourage better communication and cooperation

between the various fields and prescribers [62]. Adopting a more communicative relationship between different disciplines would not only promote a more expansive understanding of antibiotic resistance, but would also encourage more appropriate prescribing practices [62].

DRUG DEVELOPMENT

Microbial antibiotic resistance is particularly worrisome because of its ability to render our drugs useless. This unfortunate consequence means, if left unchecked, resistance could become increasingly prevalent. Perhaps we will see a day when we still have a wide choice of antibiotics, but their affect is negligible and bacterial infections that were once easy to effectively treat would become life threatening. The discovery and development of new antibiotic compounds will not solve the problem of resistance, but will provide new drugs to combat those bacterial species that are unaffected by commonly employed methods. Thus, supporting and encouraging drug development is a potential avenue for countering antibiotic resistance [38]. Unfortunately, not only has the discovery of new antibiotics lullled, but few companies are actively searching [4,8,45].

Few argue that new drugs would not provide new treatments to replace those which are faltering [38]. However, despite the benefits of drug discovery, few new compounds have ever reached market. Between 1962 and 2012, only one new antibiotic was marketed for clinical use [45]. Exacerbating this situation

is the reduction in the number of pharmaceutical companies that are actively pursuing new antibiotics. The combination of minimal success, economic environment, and regulatory barriers has greatly hindered the willingness of companies to continue to investigate new antibiotics [4,45]. Some pharmaceutical companies have opted to continue the search, but at a diminished capacity, while others, such as 18 of the larger companies, have completely withdrawn from the antibiotic hunt [4,45]. Refocusing efforts on antibiotic discovery may not be as easy as simply encouraging pharmaceutical companies to return to those activities. Multiple processes and organizations are involved in the path from discovery to clinical use. For new drugs to be developed, policies, funding, regulations, and timelines may have to be altered at the level of both companies and governments [36].

Cultivating and supporting the desire to develop new antibiotics again could impact the issue of resistance, not by halting its progression entirely, but by providing more options for treatment. Simply revitalizing the search for new antibiotics does not guarantee the discovery of more antibiotics. In response, some argue that only encouraging the practiced approaches to drug discovery will not be fruitful [8,45]. Instead, others recommend companies reemploy older methods that, when coupled with today's technology and understanding, could be highly synergistic [45]. These suggestions include returning our focus to soil

bacteria, developing more prodrugs (drugs that are administered in an inactive form and are cleaved into activation), or designing drugs that target the mechanisms of resistance [38,45]. Although these may be worthwhile approaches, discovering new antibiotics will not solve the issue of antibiotic resistance, it will only delay the progression of resistance among pathogenic bacteria.

Numerous strategies have been described and it is highly likely that more exist. These approaches vary in their focus and approach, but they have the potential to slow or prevent future occurrences of antibiotic resistance. Regardless of the method, the critical point is that individually, any one of these strategies may not have an impact on the resistance issue. Instead, it is the synergistic combination of these strategies that may alter the path of resistance. Employing a multi-faceted approach, in which patient/physician education is accompanied by guideline revision, drug discovery, and multiple other efforts, may be the only way to control antibiotic resistance [52]. As such, if we are to alter antibiotic resistance, it will likely come through multi-scale efforts employed on multiple fronts.

VII. CONCLUSION

In 1946, following the widespread use of penicillin, Alexander Fleming voiced his concerns that there would one day be a public abuse of this “miracle

drug” [4]. In the same statement, he also expressed his apprehension that such an abuse would certainly have ramifications. The inappropriate use of penicillin would not only affect the misusing individual, but would promote a resistance to that drug that could claim the lives of countless others [4]. Unfortunately, his concern was not unfounded, and the ideas behind it were not solely limited to penicillin.

While antibiotic discovery and synthesis was plentiful, it may have been difficult to imagine a future in which humans did not reign over the top of the microbial food chain. Antibiotics provided an amazing opportunity to overcome bacterial infections that previously could decimate populations. So much so, that people were confident the human race would see a day when bacterial infections were eradicated [36]. Although the discovery of antibiotics may have initially supported this ideal outlook, it has not been the result. The initial and continued use of antibiotics promotes natural selection of random mutations that allow for the development of resistance [52]. As both new and old antibiotics continue to be used and abused, resistance will persist. The issue of bacterial resistance to antibiotics has become a global concern [38]. A slew of common infections, once easily treated with antibiotic regimens, now have little to no response to drugs. *Staphylococcus aureus* is a prime example of such an infection. There was a time when antibiotics were effective against this bacterium, but now methicillin-

resistance *S. aureus* (MRSA) is prevalent in both community and in healthcare facilities. Although *S. aureus* is specifically resistant to methicillin, it serves as proof that microbial infections are able to claim lives despite the existence and availability of antibiotics. This is possible because microbes, such as this specific bacterium, have developed resistance.

There is no one individual or occupational field solely at fault for the existence of antibiotic resistance. Physicians may contribute to the problem by prescribing antibiotics for self-limiting infections or for other situations in which an antibiotic isn't beneficial or appropriate. Although it may be easy to limit the misuse of antibiotics to the healthcare industry because of a physician's access to antibiotics and their ability to prescribe them, to do so would overlook additional areas of concern. Resistance can be encouraged by patients' noncompliance, the illegal and nonprescription use of antibiotics, or even pharmaceutical companies that promote the use of one antibiotic over other equally efficacious options. The use of antibiotics in the agriculture and livestock industries may provide an opportunity for nontherapeutic levels of antibiotics to enter the environment, creating situations ideal for selecting for resistance. The increasing abundance of household products advertising antimicrobial benefits may also affect resistance in the same manner. Additionally, unhygienic conditions and failure to vaccinate set the stage for the development and dissemination of resistant strains. The

culmination of these factors, rather than any one in particular, promote and sustain antibiotic resistance.

Efforts are actively underway to prevent future antibiotic resistance and to promote the sustainable use of antibiotics. Physicians try to minimize their use of these drugs and educate themselves about regional resistant infections.

Attempts are being made to help the general public understand the issue of resistance and when antibiotic use is appropriate. In conjunction with additional methods and approaches, these efforts are an attempt to create a more knowledgeable population that uses antibiotics in an appropriate manner.

Although these practices are valuable in preventing antibiotic resistance from worsening, there remains room for improvement. Current practices should by no means be discontinued or discouraged, but new approaches should also be implemented. This includes, but is not limited to, reviving investment in antibiotic discovery and development, increasing the availability of diagnostic testing, and establishing guidelines for strict regulation of antibiotic use. Maintaining current practices and working toward future improvements may not only prevent antibiotic resistance from worsening, but may diminish it as well.

Regardless of current and even future efforts, it seems that antibiotic resistance may never be completely eliminated. Antibiotics are critical for saving lives and their application, even when used appropriately and responsibly, has

the potential to promote resistant microbes [36]. It is not practical or moral to sacrifice the health of patients for the sake of completely eradicating resistance. Considering that fact, along with the existence of organisms that harbor intrinsic mechanisms for resistance, it would be highly unlikely that the human population will ever experience a time when there is not a single drug resistant microbe [36]. However, that does not imply that the current state of antibiotic resistance cannot be improved. If action isn't taken, antibiotic resistance could potentially expand until all antibiotics are rendered useless. Under those conditions, it is possible that countless individuals could succumb to common infections that were once easily treated with antibiotics. However, encouraging and maintaining appropriate antibiotic use through multiple approaches and across numerous disciplines could improve the state of antibiotic resistance.

To keep our “miracle drugs” effective, numerous changes will have to be made. The tendency to blame others must be abandoned, as must the notion that an individual's actions won't affect the situation. It is time to stop pointing fingers, and instead, focus on making positive contributions in the fight against resistance. It is also crucial to recognize that only targeting one group or making one change will not produce desirable results. To combat antibiotic resistance, it will require a broad, multifaceted, approach that targets practices, education, and regulations at multiple levels ranging from the general public to the

government [52]. One person alone did not cause resistance, nor will a single individual be able to change it, but there is certainly the potential for each one of us to be affected by it. Thus, everyone has a part to play and it begins by understanding antibiotic resistance.

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