Mechanisms of Antibiotics and Antimicrobial Resistance: Strategies and updated technologies to reduce the possible emergence of antimicrobial resistance.

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ABSTRACT

The development of antimicrobial resistance poses an enormous threat as it renders antibiotics ineffective against pathogens, making previously treatable conditions harder to control. The World Health Organization estimates about 700,000 people die due to multidrug-resistant diseases annually. This number is expected to steadily increase at an alarming rate over the next few decades. Thus, antimicrobial resistance should be better understood to help improve these statistics. This review outlines the major mechanisms of antimicrobial resistance, explains how the microbe gains this resistance (dissemination of antimicrobial resistance), and explores methods of combating antimicrobial resistance in the future.

1.INTRODUCTION

Antimicrobial agents are chemical substances that suppress or destroy microorganisms, including pathogenic bacteria. Penicillin, the first significant antibiotic, was discovered in 1928 when Alexander Fleming found that penicillium mold killed bacteria. Using Fleming's discovery, Howard Florey created a usable, purified form of penicillin in 1940 (American Chemical Society). During this time, multiple new antibiotics were discovered, including their biochemical structures, and target specificity. For example, streptomycin was introduced in 1944 was widely used for treatment of tuberculosis (TB) (Woodruff). Since then, scientists have discovered numerous antibiotic classes which use different methods to suppress bacteria. However, since the discovery of antibiotic-resistant plasmids in the 1960s, increasing antibiotic resistance has also been documented (Davies).

Since antimicrobial agents were first implemented, numerous bacterial pathogens have evolved or acquired drug resistance. For example, M.tuberculosis has evolved to resist streptomycin which had previously shown to be highly effective (Davies). The use of other anti-TB treatments were also initially successful, but ultimately led to further antibiotic resistant mutations, producing highly drug-resistant and sometimes totally drug-resistant strains (Davies).

This paper will analyze three key mechanisms of antimicrobial resistance, including enzymatic degradation of antibacterial drugs, alteration of bacterial receptor proteins, and changes in membrane permeability. Additionally, the paper will discuss the evolutionary perspective of the two genetic approaches used by bacteria to combat these mechanisms and adapt to the antibiotics, including gene mutation and horizontal gene transfer. Overall, this work seeks to conduct an in-depth analysis of the basic fundamental mechanisms of antimicrobial resistance, and review the current methods of reducing this resistance.

2.ANALYSIS OF THE FUNDAMENTAL MECHANISMS OF ANTIMICROBIAL RESISTANCE

There are three key mechanisms of antimicrobial resistance: enzymatic degradation of antibacterial drugs, alteration of bacterial proteins, and changes in membrane permeability. The following is an example of resistance mechanisms employed by bacteria against penicillins and cephalosporins.

2.1 Enzymatic degradation of antibacterial drugs.

A common defense bacteria use against antimicrobials is modifying the antimicrobial compound using specific enzymes. The enzymes modify or degrade antimicrobial agens which then render them unable to disrupt any processes and therefore protect the bacteria from destruction (Munita). For example, aminoglycoside antibiotics, which include streptomycin, are one group one group of commonly resisted antibiotics that are degraded by bacterial enzymes (Ramirez). Aminoglycosides work by binding to anionic sites on the cell membrane, which causes increased permeability of the outer

bacterial membrane. This allows aminoglycosides to enter the periplasmic space between the outer bacterial membrane and the inner membrane (Ramirez). Once in the periplasmic space, a small number of aminoglycosides can enter the cytoplasm, which interferes with protein synthesis. This interference leads to more deficiencies of the cytoplasmic membrane and therefore an increased intake of aminoglycosides, resulting in the death of the bacteria (Ramirez). However, within some bacteria, aminoglycoside modifying enzymes catalyze the degradation/modification at the links that hold the structure of streptomycin (see fig. 1), without which the aminoglycoside antimicrobials cannot fully function, making it unable to interfere with protein synthesis (Ramirez).

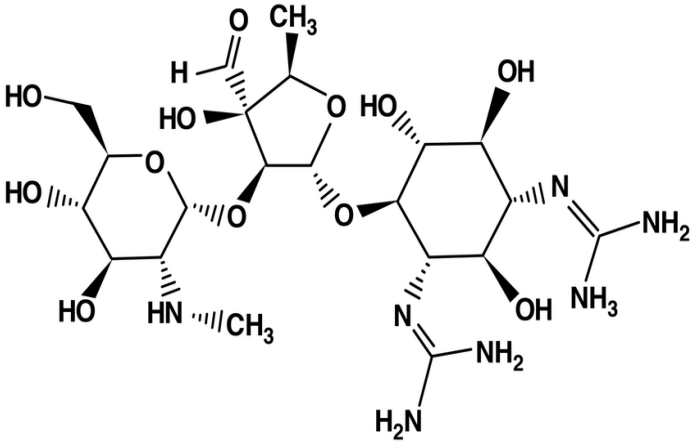

Figure 1. The structure of streptomycin, a commonly used aminoglycoside antibiotic. Figure taken from Bryrida Kwiatkowska et al., "Immune system as a new therapeutic target for antibiotics," *Advances in Bioscience and Biotechnology*, vol. 2013, no. 4, pp. 91-101, www.researchgate.net/publication/266202548_Immune_system_as_a_new_therapeutic_target_for_antibiotics.

Although the mechanism for antimicrobial resistance in streptomycin resistant *Streptomyces* species have yet to be fully elucidated, the presence of modification enzymes and loss of efficacy of streptomycin is well correlated. (Peterson and Kaur, 2018). In *Streptomyces griseus*, a modification enzyme phosphorylates streptomycin to streptomycin-6-phosphate, an inactive precursor that cannot bind to ribosomes, and therefore cannot interfere with protein synthesis (Shinkawa).

2.2. Alteration of bacterial proteins that are antimicrobial targets.

Another strategy bacteria employ for antimicrobial resistance is alteration of bacterial proteins that are targeted by antimicrobial agents such that the antimicrobial agents are unable to bind to the bacteria (Munita). Most antimicrobial agents work by binding to and disabling proteins that are essential for the bacteria's life cycle (Munita). Thus, the inability to bind to the target effectively prevents the antimicrobial agent from having an effect (Munita).

For example, beta-lactam antibiotics destroy bacteria by binding and inactivating penicillin-binding proteins (PBP) (Fong). PBPs are necessary for cross linking the bacterial cell wall, and are crucial to effectively maintaining the structure of the peptidoglycan layer. The peptidoglycan layer is a chain of polysaccharide that form a net-like structure around the cell membrane of certain bacteria to maintain cell shape and provide resistance against internal pressure (Vollmer). Thus, when PBPs are inactivated by penicillin or other Beta-lactam antibiotics, the cell is unable to synthesize a new cell wall when dividing, leading to cell death by osmotic rupture resulting from the difference of solute concentration inside and outside of the cell (Cho).

Bacteria gain resistance to Beta-lactam antibiotics through changes in the PBP that decrease the affinity of Beta-lactam antibiotics to the binding sites. For example, for *S.aureus* bacteria, mutations in the PBPs result in the expression of a PBP2a enzyme instead of PBP on the outer surface of the bacteria (see fig. 2) (Santajit). PBP2a has low affinity with Beta-lactam antibiotics, and thus allows for bacteria with these targets to survive in the presence of penicillin (Santajit).

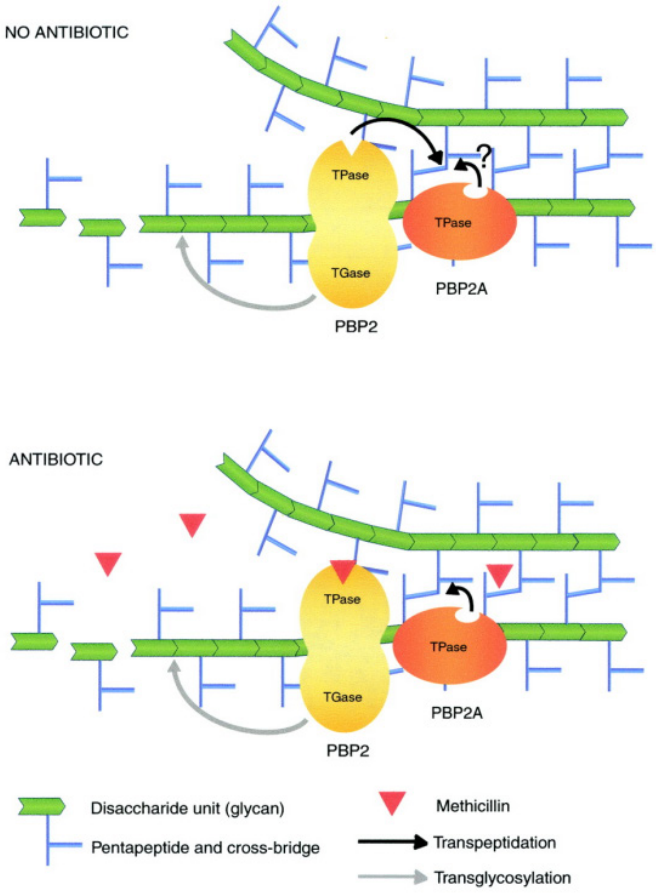

Figure 2. Bacterial transmembrane protein PBP2A and how it reacts to presence of methicillin (Beta-lactam antibiotic). Figure taken from Mariana G. Pinho et al., "An acquired and a native penicillin-binding protein cooperate in building the cell wall of drug-resistant staphylococci", *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 19, pp. 10886-1089, https://www.pnas.org/content/98/19/10886.

2.3 Changes in membrane permeability to antibiotics.

Another method of antimicrobial resistance is to change the permeability of antibiotics in the outer membrane of a gram-negative bacteria. Most antimicrobial agents work by interfering/disabling inner workings of bacteria. In order to do so, antimicrobials first have to reach the cytoplasm, penetrating the outer membranes that protect bacterial cytoplasm from outside (Munita). Changing the membrane permeability can therefore prevent antimicrobial agents from reaching their targets.

The outer membrane of gram-negative bacteria is an asymmetric bilayer of phospholipid and lipopolysaccharides, which makes it able to block the passage of most hydrophobic and hydrophilic molecules (see fig. 3) (Delcour). Thus, the outer membrane is only penetrated using two specific paths depending on the polarity of the antibiotic. Hydrophobic antimicrobial agents must pass through a lipid mediated pathway, while hydrophilic antimicrobials must pass through diffusion porins located on the outer membrane, which are size restrictive (Delcour). For example, Beta-lactam antibiotics are able to go through the pore-forming proteins since they are small and hydrophilic (Delcour). Changes in proteins embedded in the outer membrane directly relate to antimicrobial resistance and therefore, the composition of the bacterial outer membrane affects the sensitivity of that bacteria to various antibiotics (Delcour).

Accordingly, decreased expression of porin molecules, by mutation or environmental factors, causes decreased efficiency of antimicrobial agents that have to enter the bacteria through porins, leading to drug resistance (Ghai). For example, a mutation which led to decreased expression of membrane protein Omp was shown to contribute to imipenem and meropenem (types of antibiotics) resistance (Ghai).

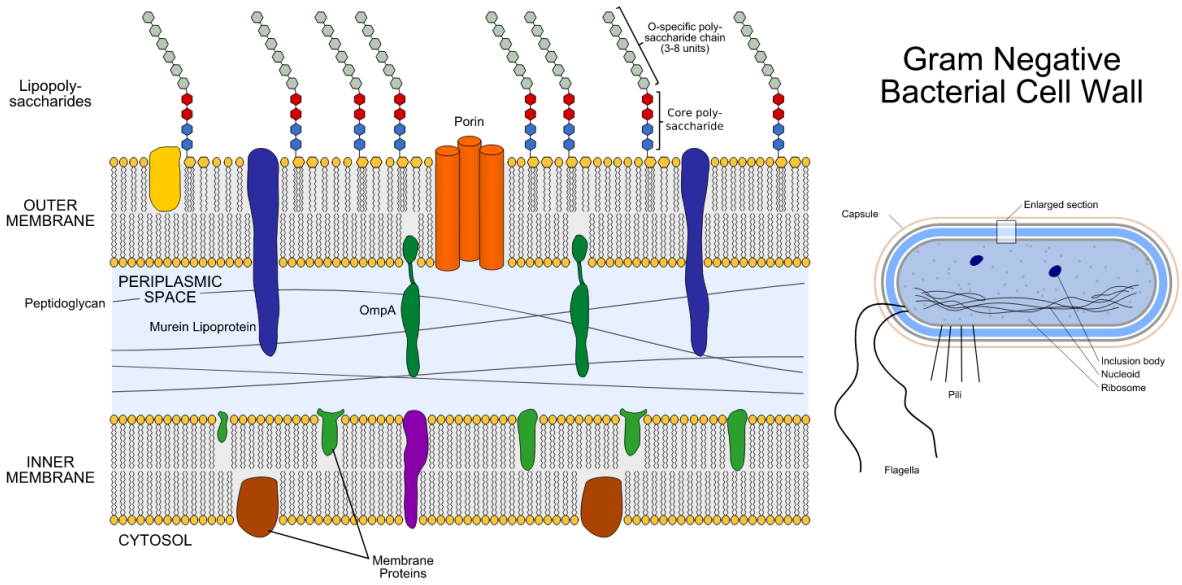

Figure 3. Structure of the gram-negative bacterial cell wall. Figure taken from *Structure of Gram-negative Cell Wall*, *LibreTexts*, bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(Boundless)/4%3A_Cell_Structure_of_Bacteria%2C_Archaea%2C_and_Eukaryotes/4.4%3A_Cell_Walls_of_Prokaryotes/4.4B%3A_Gram-Negative_Outer_Membrane.

3. GENETIC BASIS OF ANTIMICROBIAL RESISTANCE

Bacteria mechanisms of antimicrobial resistance are developed through mutation. According to Munita and Arias (2016), bacteria have incredible genetic plasticity, enabling them to respond effectively to a wide assortment of environmental threats, such as the presence of antibiotic molecules that might jeopardize their existence (Munita). This section will present a discussion on the evolutionary perspective of the two genetic approaches used by bacteria to adapt to the presence of antibiotics.

Most antimicrobial mechanisms are based on genetic

mutations that cause antimicrobial agents to be unable to reach their target(s) intact. Enzymatic degradation of antimicrobial molecules, alteration of target protein, and change in outer membrane permeability usually require some type of mutation (Munita). The method in which the bacteria acquires the mutations is primarily through two pathways (Munita).

3.1 Mutation resistance.

The first, and most intuitive method in which bacteria gain antimicrobial resistance is through random mutations. Once an antimicrobial agent is used, it eliminates all susceptible bacteria. However, approximately one in every billion bacteria will have developed a mutation that restricts antimicrobial action (Woodford). The insusceptible bacteria, or the ones that by chance mutated to be resistant, will survive the antimicrobial agents. These mutations may include mutations in the outer membrane or the production of enzymes that can degrade the antimicrobial agent. When susceptible bacteria die, they leave space and resources for the resistant strains of bacteria to reproduce (Munita). Eventually, the bacteria with resistance to that specific antimicrobial agent will grow to repopulate the environment (see fig. 4). When the same antimicrobial agent is used again, most of the bacteria will now be resistant to that agent (Munita).

For example, fluoroquinolones are antibiotics that kill bacteria by targeting two different types of topoisomerases, which are crucial bacterial enzymes that catalyze the supercoiling of double-stranded closed-circular DNA (Fluoroquinolones) and are necessary for bacterial DNA replication. When fluoroquinolones are used on gram-negative bacteria, they attack the target enzymes and effectively kill the bacteria. However, some strains of bacteria have gained resistance to fluoroquinolones by accumulating mutations in DNA gyrase (a type of topoisomerase) (Jacoby), resulting in reduced susceptibility to fluoroquinolones. Additionally, the resistance can be enhanced further by other mutations that limit the ability of the antimicrobial to enter the cell (Jacoby). This strain of gram-negative bacteria can then go through fission and repopulate, with the entire new population now resistant to the fluoroquinolones that were used before (Jacoby).

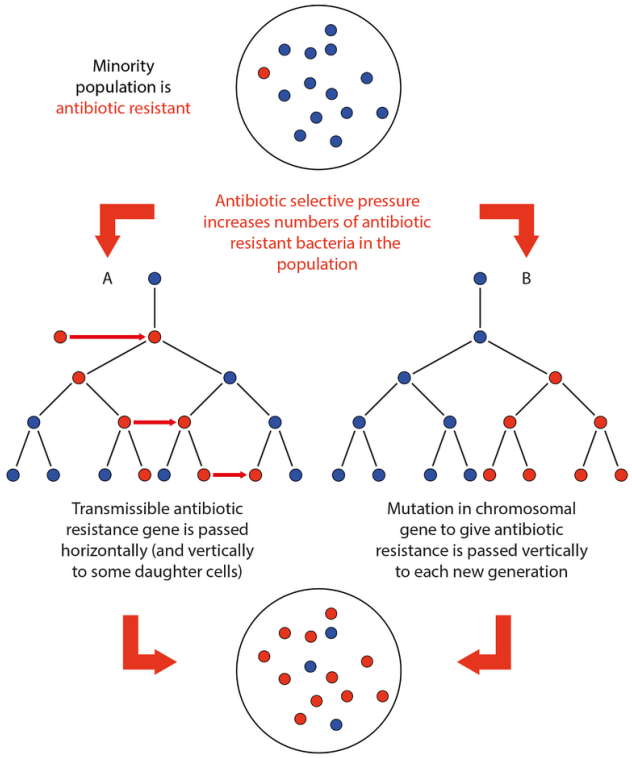

Figure 4. Impact of antimicrobial agents on rate of vertical transfer of antibiotic resistance. Figure taken from Richard William Meek et al., An illustration of how antibiotic resistance is selected in a bacterial population and how it proliferates, *PLOS Biology*, 7 Oct. 2015, journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1002266.

3.2 Horizontal gene transfer.

In addition to vertical transfer of antimicrobial resistance through bacterial reproduction, bacteria can gain the necessary genes for antimicrobial resistance in other ways. The acquisition and incorporation of outside genes into the genome is called horizontal gene transfer (HGT) (Clark). This method of gene transfer is "the most fundamental drivers of evolution that is frequently responsible for the development of antimicrobial resistance" (Munita). Horizontal gene transfer occurs with three major mechanisms.

The first way bacteria can acquire outside genes is through is transformation (see fig. 5). Transformation is when bacteria absorb short DNA fragments on the outside of the cell (Clark). The requirement for this type of gene transfer is that the cell that is in a state of competence, meaning it is able to absorb nearby DNA fragments (Wintersdorff). Although meeting these conditions is unlikely in the natural environment, transformation of resistance genes still has been observed

in the development of antimicrobial resistance to fluoroquinolones where resistance genes were readily tranformed between bacteria from Streptococcus genus (Wintersdorff).

The second method of horizontal gene transfer is through transduction, where a bacteriophage (a virus that infects bacteria and parasitizes it) transfers genes from one bacterium to another (Clark) (see fig. 5). Bacteriophages normally infect bacteria by releasing their own DNA into the bacteria and replicating inside of them. Usually, the bacteriophage kills the bacteria by making it lyse, or burst, releasing more bacteriophages to infect more bacteria. However, there are cases when the bacteriophages "mistakenly incorporate a piece of the bacterial DNA into a phage head in place of phage DNA." (Griffiths) This mistake by the phage causes the next infected bacteria to gain the genetic material of the previous bacteria instead of the phage DNA (Griffiths). Unlike the phage DNA, it is more likely that the foreign DNA is very similar to the chromosomal DNA, making it more likely to be successfully incorporated (Griffiths). Thus, the host does not try to degrade this chromosomal sequence, but rather keeps it as a plasmid or incorporates it. Transduction of antimicrobial resistance has not been thoroughly studied, yet scientists estimate transduction to be a significant method of resistance transfer (Wintersdorff).

The last method of horizontal gene transfer that has been important in the rapid development of antimicrobial resistance in clinical settings is conjugation (see fig. 5). Also known as bacterial sex, conjugation is a very efficient way for bacteria to transfer genetic information directly by a process using cell surface pili (a thin fiber tubules that can be used for attachment of bacterial cells whose tip has an adhesin, a protein that can attach itself to other surfaces) (Atlas of Oral Microbiology) (Wintersdorff). Conjugation involves cell to cell contact and the transfer of mobile genetic elements, such as plasmids and transposons (Hoek). Conjugation is a driving factor of increasing antimicrobial resistance in hospital settings and it is known to occur in high frequency in the gastrointestinal tract of patients under antibiotic treatment (Munita).

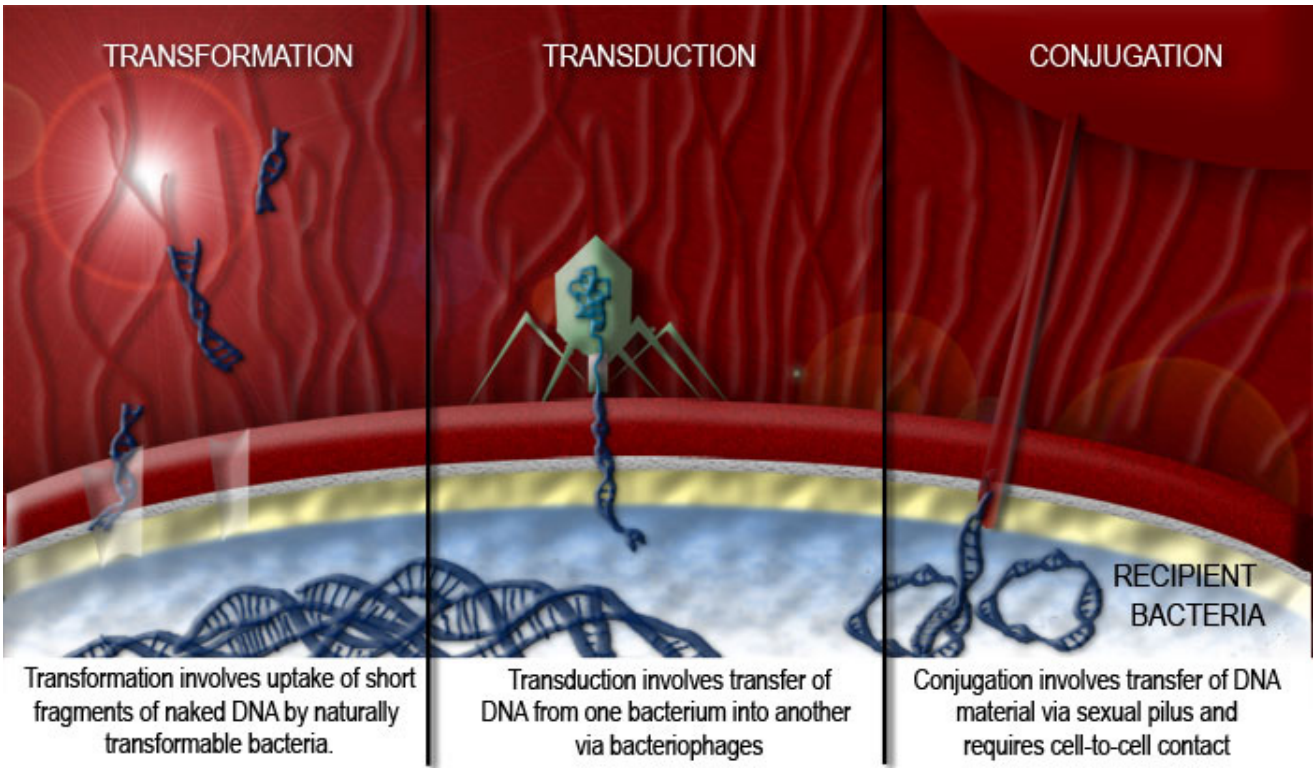

Figure 5. Illustration of the three main mechanisms of horizontal gene transfer: transformation, transduction, and conjugation. Figure taken from *Horizontal Gene Transfer*, *Antimicrobial Resistance Learning Site: Microbiology*, Michigan State University, amrls.cvm.msu.edu/microbiology/molecular-basis-for-antimicrobial-resistance/acquired-resistance/acquisition-of-antimicrobial-resistance-via-horizontal-gene-transfer.

4. STRATEGIES AND UPDATED TECHNOLOGIES TO REDUCE THE POSSIBLE EMERGENCE OF ANTIMICROBIAL RESISTANCE

Through the mechanisms enumerated above, antimicrobial resistance has continued to pose a greater threat to global health. For example, a new strain of *acinetobacter* (bacteria that causes pneumonia) has emerged (CDC). This strain is able to resist Carbapenem, a previously highly effective antimicrobial agent that is used for suspected multidrug resistant strains of bacteria (CDC). In addition to the new strain of *acinetobacter*, the Centers for Disease Control and Prevention (CDC) lists an enormous number of bacteria that have acquired resistance against previously effective antimicrobial agents. These data highlight the severity of the threat of antimicrobial resistance to global health.

Though bacterial resistance is acquired and transferred through vertical and horizontal gene transfer, there are strategies to mitigate the degree of the mutation and evolution of antimicrobial resistance. Current available strategies mostly pertain to regulations regarding the use of antimicrobial drugs, but there are some emerging technologies that could heavily reduce the threat of antimicrobial resistance. This section will outline the regulations in place for the usage of antimicrobial agents and explore two novel biological solutions to antimicrobial resistance currently being developed.

4.1 Regulations regarding the usage of antimicrobial agents to prevent antimicrobial resistance.

The first and most intuitive method to reduce antimicrobial resistance is to follow professional guidelines in the usage of antimicrobials when treating infected individuals (fig. 6). For example, antimicrobial drugs have tiers depending on the severity of the infection. Ignoring such guidelines may lead to an increase in resistance to all antimicrobial drugs (Uchil). Using established regimens for antibiotics in high risk cases for the shortest duration possible can minimize the risk of developing antimicrobial resistance (Uchil).

However, in developing countries, "less than 40% patients in public sector and less than 30% patients in private sector are treated in accordance with standard treatment guidelines". This means that a large number of people are using antimicrobial drugs without following professional guidelines, often resulting in the development of resistance to the antimicrobial used (Uchil). Education about antimicrobial resistance as well proper administration of antimicrobial drugs can be crucial in suppressing the development of antimicrobial resistant strains of bacteria.

In addition to the rational usage of antimicrobial agents, another aspect to consider is communicability of the resistant strains of bacteria, particularly in hospital environments (CDC). When antimicrobial resistant bacteria infect a large number of people, this results in widespread dissemination of antimicrobial resistance (CDC). Thus, a high standard of hygiene and infection prevention is required in order to contain antimicrobial resistance (CDC).

Antimicrobial resistance is also commonly observed in the agricultural industry. Globally, antimicrobial drugs are used in agriculture, where farm animals, such as cows, pigs, and chickens, are often treated with high dosages, as usage in animals is far less regulated when compared to use on humans (see fig. 6). For example, in the early 2000s, farmers would routinely use antimicrobial drugs like avoparcin to address unsanitary conditions in which pork was produced. This significantly contributed to the increase in avoparcin-resistant bacteria (Martin). After Denmark, one of the main exporters of pork, promptly banned the use of avoparcin in 2006, and since then, the level of avoparcin-resistant bacteria has remained low (Martin). Other than the development of antimicrobial resistance in the animals themselves, unrestrained use of antimicrobials leads to other problems, such as the spread of resistance through waste (Rousham). For example, chicken waste and unused internal organs are often used to feed aquaculture, where the antimicrobial resistance can pass to fish (Rousham). Regulations banning or limiting the use of antimicrobial drugs and research on alternatives are necessary in order to slow the development of antimicrobial resistance (Rousham).

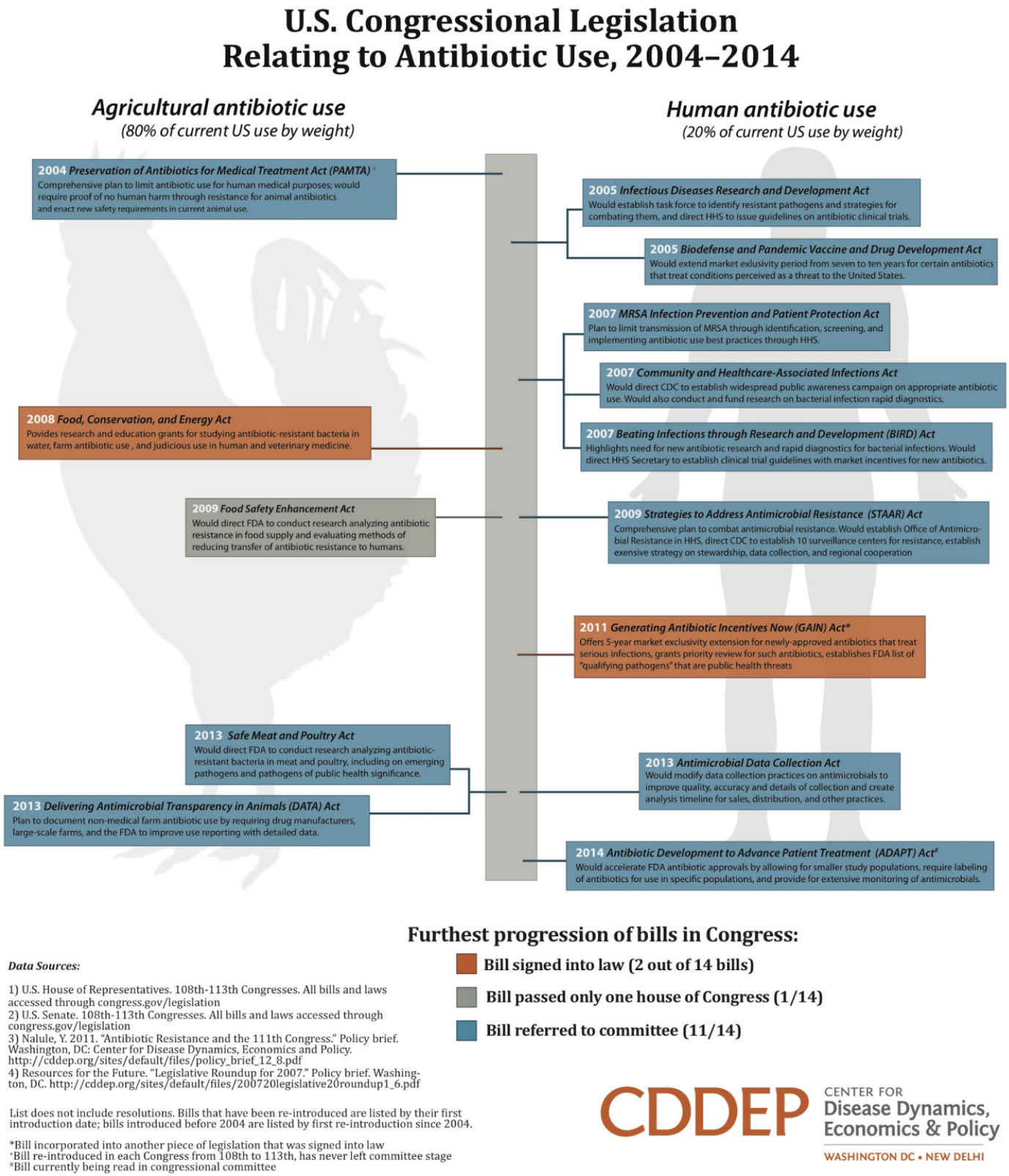

Figure 6. Historic laws on medical and commercial (agricultural) antimicrobial usage in the United States. Figure taken from "Congress Legislation Relating to Antimicrobial Use, 2004-2014," *The Center For Disease Dynamics, Economics & Policy*, cddep.org/tool/us_congressional_legislation_relating_antibiotic_use_2004_2014/.

4.2 Technologies to combat antimicrobial resistance.

In order to address the quickly emerging threat of antibiotic resistance, researchers have been developing innovative approaches to combat antimicrobial resistance. This section will highlight two emerging strategies to combat antimicrobial resistance.

One emerging method is potentiation, or the use of two different substances to create a synergistic effect (Baker). An example of this method is the use of superoxides with other antimicrobial agent. Superoxides are oxygen molecules that have an extra electron and are a type of the reactive oxygen species, which are chemically reactive substances that cause damage in cells. Because of their chemical reactivity, superoxides can cause oxidative stress, in which detrimental chemical reactions within the cell lead to damage or death of the cell. This includes damage the outer membrane of gram-negative bacteria, which weakens the barrier effect of the bacterial outer membrane (Courtney). According to a study by Courtney, when superoxides were used in combination with various antibiotics, it increased the sensitivity of the bacteria to those antibiotics, reducing the required amount of antibiotics by up to 1000 fold (Courtney). Another example of potentiation is the use of efflux pump inhibitors alongside antimicrobial agents. Most bacteria are able to resist the effect of antimicrobial drugs by actively pumping the drug out of the cell, effectively reducing the concentration of drug in the bacteria to survivable levels (Baker). By inhibiting the efflux pumps, the antimicrobial drugs can have increased potency and range of effectiveness (Baker). Although research on employing efflux pump inhibitors is ongoing, no conclusive progress has been shown in the past three years (Baker).

Another important technology in combating antimicrobial resistance has been the use of vaccines (Jansen). Vaccines function by exposing and training the human immune system to recognize antigens, which are fragments of the bacteria (or virus). Vaccination triggers an immune response, which then trains the immune system to recognize that antigen and induce an immune response apon secondary recognition of that antigen. In this manner, vaccinated individuals may be protected from infection, preventing the need for antimicrobial drugs. Vaccines have proven to be a crucial tool to lessen the overall use of antimicrobial drugs (WHO) (see fig. 7). Vaccines not only prevent bacterial or viral infections (like influenza), but it also aids in the body's ability to fight off secondary disease (like pneumonia) after the primary infection weakens the immune system (WHO), thus reducing the need to prescribe an antimicrobial drug. Historically vaccines have been very effective in reducing usage of antibiotics (Jansen).

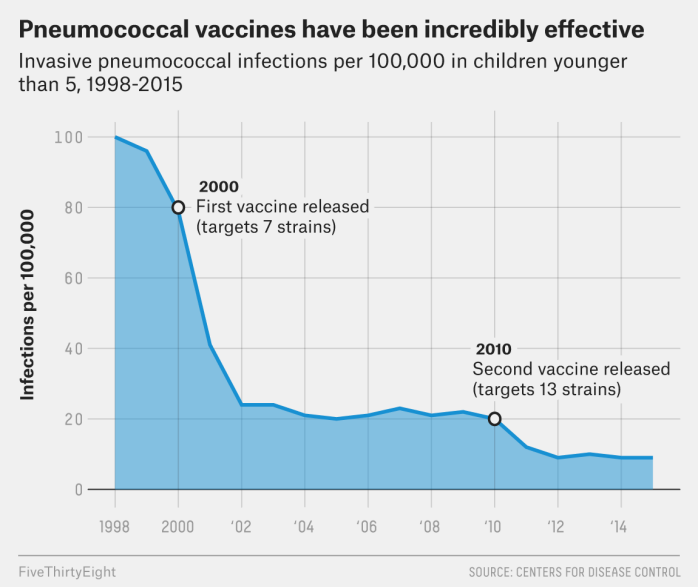

Figure 7. Vaccines effectively decreases infection rate. Figure taken from Alice Callahan et al., "Pneumococcal Vaccines Have Been Incredibly Effective," *Five Thirty Eight*, ABC News, 15 Aug 2017, fivethirtyeight.com/features/more-vaccines-fewer-antibiotics/.

5. CONCLUSION - LOOKING FORWARD

Currently, the World Health Organization states that there are at least 700,000 people who die as a result of disease caused by antimicrobial resistant infections. They also estimates that there could be 10 million deaths each year due to antimicrobial resistance by 2050 (WHO). Thus, combating antimicrobial resistance is critical for public health.

Currently, the solutions against antimicrobial resistance are incomplete and insufficient. Stricter regulations on the use of antimicrobials have limits, as these guidelines may not be globally applicable. Current technologies for combating antimicrobial resistant bacterial strains also have significant limitations. Although effective in the short-term, the prevalence of multidrug resistant strains of pathogenic bacteria has been rising, meaning that drugs today may soon become ineffective (Nikaido).

Vaccines also have limitations. By their very nature vaccines are preventative, meaning that vaccines do not treat active infections. Moreover, vaccine development is not necessarily straightforward, and can require significant development time, meaning that they may not always be available for every pathogen. Even when vaccines are available for a certain pathogen, they are often expensive to develop, produce, and preserve and so ultimately the price per dose is too high to

be available around the world. For example, effective vaccines have been developed to fight *S. pneumoniae*, a pathogen which is responsible for about 30 percent of pneumonia and attis media, causing 1.2 million deaths yearly (Vaccines For Amr). However, global coverage remains low at around 40 percent due to the cost per dose, especially in China and India (Vaccines For Amr).

Going forward, it is imperative that many resources are invested to effectively combat antimicrobial resistance. As scientists learn more about novel ways to overcome antimicrobial resistance mechanisms, our technologies and regulations must keep pace with the development of antimicrobial resistance.

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