Chapter 1: Antibiotics and Antibiotic Resistance

1.1 Introduction

For a treatment to be considered an **antibiotic** it must include two critical features: It must stop or kill bacterial growth, and it must not harm the host. The **“Golden Age of Antibiotics”** begin in the 1950s and lasted until the 1970s and consisted of the discovery of many new antibiotics, the first being Penicillin in 1928. However, Penicillin did not become commercialized until 1945. In March of 1942 **Anne Miller** was the fifth person to be treated with Penicillin, but most importantly she was also the first person to be completely cured by it. This was an important milestone for Penicillin.

On November 28th, 1942 an unfortunate event called the **Cocoanut Grove Fire** occurred at a night club in Boston. Cocoanut Grove was an overcrowded nightclub that caught on fire on the inside and did not have any fire exits or large enough doors for everyone to get out. As a result, 492 people died and roughly 130 others were sent to the hospital with non-fatal injuries. Just weeks before the fire, the New York Times wrote an editorial about how Penicillin was a great option that could be used to treat many people. This became ironic because after the fire occurred, there was not nearly enough Penicillin to treat everyone and those who were able to receive the Penicillin were not given enough to actual heal their infection. The devasting fire created a need for fire safety laws and it also created a rush for pharmaceutical companies to find new ways to quickly manufacture Penicillin.

 **Antibiotic resistance** was first observed in 1947 against Penicillin and has been a problem for most antibiotics ever since. Antibiotic Resistance is defined as the ability of a bacterial cell to continue to grow in a concentration of a substance that would normally stop/kill bacterial cell growth. Over time, bacterial cells have mutated to become resistant to antibiotics. However, mutation is not the only cause. Cells can be innately resistant or they can acquire their resistance mechanisms.

1.2 Common Uses for Antibiotics

 Antibiotics have multiple different uses. The most commonly known use is that we use them to treat infections when we get sick. Usually when someone begins to feel sick they go to the doctor where tests can be performed to determine what kind of infection you might have and what medicine would work best to treat the specific bacteria that is causing your infection.

 The most common use for antibiotics worldwide is actually in agriculture and used for food production. **Growth promotion** occurs when farmers give their animals small doses of antibiotics in order to make them grow bigger very quickly and with less feed so that they can be sold to the market quicker. The use of the antibiotics also caused the animal to have a smaller percentage of fat and a higher percentage of protein in the meat. The subclinical doses also allowed for the animals to be protected from infectious disease which meant that the farmers could keep them in a smaller space with a small chance of them getting sick and infecting the other animals.

 However, in 2015 A national action plan titled the Veterinary Feed Directive called for antibiotics in animal feed to only be used for reason such as treating disease, controlling the spread of disease, and for surgical or other medical procedures. This was created in order to decrease the rate of resistance.

1.3 Antibiotic Classes

 So far, we have discovered a very wide range of antibiotics and this is great because there are so many different species of bacteria that can cause infections. There are many reasons that is important for us to have this wide range of antibiotics. One of the most common reasons is that people can have allergies to some of the antibiotics and so they need other options. Another reason is that some variants of the same antibiotic can have a different potency level meaning that some could work for longer and be resistant to break downs over time allowing them to be stored for longer.

 There are so many different classes for antibiotics that have the ability to treat different things. Some of these classes are used for clinical reasons, for example some of these classes are known as beta-lactams, aminoglycosides, macrolides, streptogramins, tetracyclines, sulfa drugs, and fluoroquinolones. All of these classes can be used to treat different types of infections depending on the bacteria that is present. There are even some classes that are used for non-clinical reasons such as agriculture.

1.4 Antibiotic Resistance

 **Innate Resistance**, otherwise known as Genetic Resistance, occurs within bacterial cells that are already antibiotic producers. Since the bacteria itself is producing the antibiotic, it has to be able to protect itself against the antibiotic’s effects. These cells will typically use efflux pumps or target modification as defense mechanisms to ensure that it is not negatively affected by the antibiotic that it is producing.

 **Acquired Resistance**, otherwise known as Non-genetic Resistance, occurs when a bacterial cell mutates to be able to resist antibiotics or is resistant through another method. Two methods that do not require mutations are the formation of a biofilm, and persister cells. A **biofilm** is a self-produced matrix that a bacteria can produce outside of the cell in order to protect itself. The biofilm is hard for the antibiotic to penetrate so this allows for easy resistance. **Persister cells** are cells that lie dormant and are non-growing in an antibiotic solution. These cells can be inside of the biofilm and so that allows for them to be protected from the antibiotic solution which allows for them to grow again once they are out of the antibiotic solution.

There are two main ways that bacterial cells can gain a mutation to cause resistance to antibiotics, horizontal gene transfer and vertical transmission. In **horizontal gene transfer,** cells can gain resistance through mutation, conjugation (bacterial sex), competence (DNA up-taking), and through phage transduction. In **vertical transmission**, cells can gain a point mutation in their RNA that causes them to become an antibiotic resistant subpopulation. Since the subpopulation is now resistant to the antibiotic, when exposed the non-resistant cells will die off and the new resistant subpopulation will grow to take over.

 One of the most important ways to differentiate bacteria is to figure out if the cell is either **gram-positive** or **gram-negative**. This is important because the structure of the cell wall is different depending on which classification it is. The structure of a cell that is gram-positive consists of two layers - a thick cell wall and an inner cytoplasmic membrane. The structure of a gram-negative cell consists of three layers - an outer membrane layer, a thin cell wall, and an inner cytoplasmic membrane. This difference in bacteria itself also plays a factor into how well the antibiotics work. Typically, organisms that are highly resistant are gram negative because their cell walls are harder to penetrate since it is made up of three layers instead of two.

Bacteria are ubiquitous, meaning they are everywhere – even located on and within the body. The body’s microbiome consists of multiple species of **commensal** **bacteria** that act as a protective barrier to help prevent you from getting sick. **Pathogenic bacteria** are bacteria that can cause disease in completely healthy organisms and immunocompromised organisms. Another kind of pathogen is an **opportunistic pathogen**, these types of pathogens are bacteria that normally do not cause you to become sick, but if you have a compromised immune system then they can cause you to get sick. There are some species of bacteria that are infamous for being resistant to antibiotics and these are known as the **ESKAPE Pathogens**:

**E**nterococcus faecium

**S**taphylococcus aureus

**K**lebsiella pneumoniae

**A**cinetobacter baumannii

**P**seudomonas aeruginosa

**E**nterobacter spp.

The ESKAPE Pathogens have been identified by WHO (The World Health Organization) as bacteria that it is top priority to start finding or creating antibiotics that the bacteria are still susceptible to.

However, the mechanisms that these bacterial cells use to be resistant to antibiotics are complex. There are four main defense mechanisms that the cells utilize for resistance. These mechanisms are:

1. Efflux Pumps
2. Porin Modification
3. Target Modification
4. Destruction/Modification of the Antibiotics

1.5 Efflux Pumps

 **Efflux pumps** reside on the cell wall and are used to actively pump the antibiotics out of the bacterial cytoplasm and membrane. There are numerous types of efflux pumps because they can be either specific or general. **Specific efflux pumps** are pumps that will only transfer whatever molecule is it is specific for such as an antibiotic like Tetracycline or Chloramphenicol. **General efflux pumps** will transfer multiple molecules across the membrane such as biocides, disinfectants, toxins, and antibiotics.

 Efflux pumps can be powered through different sources of energy – they can use the proton motive force, or ATP. The **proton motive force** is the force that is used to pump protons across a cellular membrane in the direction of the downhill electrochemical gradient. Adenosine Triphosphate, or **ATP** is a high energy molecule that is found in every cell and is used as an energy currency. This is another place where the difference between gram-positive cells and gram-negative cells is important. Since the gram-negative cell wall is bigger than the gram-positive cell wall, the pumps sometimes have to be bigger so they can stretch across both the inner membrane and the outer membrane. The pumps can also be classified into five different groups:

1. Multidrug and Toxic Compound Extension (MATE)

2. Major Facilitator Superfamily (MFS)

3. Small Multidrug Resistance (SMR)

4. Resistance Nodulation Division (RND)

5. ATP Binding Cassette (ABC Superfamily)

**MATE pumps** are powered by a proton motive force and are found in gram positive bacteria. These pumps are used to pump out specific antibiotics. **MFS pumps** are powered by the proton motive force and can be found in both gram-positive and gram-negative bacteria. When found in gram-negative bacteria, the pump is larger and has an outer membrane component attached. These pumps can be used to pump out both general classes of antibiotics, and disinfectants. **SMR pumps** are powered by the proton motive force and can be found in both gram-positive and gram-negative bacteria. These pumps are small, as the name implies, and likely function as multimers. They can be used to pump out disinfectants. **RND pumps** are powered by the proton motive force and are found in gram-negative bacteria. These pumps are incredibly important in gram-negative organisms because they are a much larger pump that allows molecules to be pumped across both the inner and outer membrane. They are also able to pump across the most kinds of molecules such as general classes of antibiotics, antibiotics with large molecules, biocides, and virulence factors. **ABC Superfamily** **pumps** are the only pumps powered by ATP and can be found in either gram-positive or gram-negative bacteria. These pumps are also used for specific antibiotics.

1.6 Porin Modification

 **Porin** is a type of protein produced by bacterial cells in the cell wall that functions by forming a channel that is large enough to allow the passage of small molecules through cell membranes. When this protein is modified, it typically will not form a channel anymore which causes the cell wall to be less open to antibiotic molecules meaning that they can no longer penetrate the bacteria and affect it. Since the antibiotics can no longer penetrate the bacterial cell, they are no longer effective and the cell is now resistant.

1.7 Target Modification

 There are multiple ways that **target modifications** can occur. The main modifications that are made are point mutations, changes to the RNA, and

 When a point mutation occurs, there is one codon in the RNA that is changed that can now allow for the bacteria to be resistant to the antibiotic. Once this begins, the cell will continue to pass along the mutation through vertical transmission which allows for the subpopulation of the antibiotic resistant cell to continue to grow while the non-resistant cell dies.

 When a larger change to the RNA is made, it can modify an entire binding site on a cell. These types of modifications are advantageous to the bacterial cell because it is still a modest modification, meaning that it is not lethal and would not kill the bacteria. A common way that this can occur, is when cells undergo methylation. This occurs when a methyl group is added onto a molecule. The addition of this methyl group could change the way a typical binding site on a bacterial cell works and could block the antibiotic from being able to bind to the cell at all. Since the antibiotic can no longer bind to the cell, it will not be able to kill it or stop the growth of it and the original bacterial cell is not resistant to the antibiotic.

1.8 Destruction or Modification of an Antibiotic

 There are many different classes of antibiotics. One of the classes that this section will focus on is **Beta-Lactams**. An example of a beta-lactam antibiotic is Penicillin.

These kinds of antibiotics produce an enzyme known as a **Beta-lactamase** that allows for them to be able to resist antibiotics. These enzymes can be inhibited by molecules that are called **Beta-lactamase inhibitors**. There are four different classes of Beta-lactamase inhibitors, known as Class A, Class B, Class C, and Class D. The differences between these classes vary among the active sites of the molecule, what they work against, and what inhibits them.

 One of the interesting features of beta-lactamase inhibitors is that they can actually still be used as an antibiotic when they are paired with their inhibitors. This is known as **combination therapy** because the pairing of the antibiotic inhibitor and another inhibitor allows for the antibiotic to no longer be blocked.

 **Antibiotic Inactivation** can occur when a cells enzyme is modified so that it can react with the antibiotic molecule so that it no longer affects the microorganism. Typically, this modification occurs when another chemical group is added onto an existing molecule. The three main processes of this are acetylation, phosphorylation, and adenylation. When acetylation occurs, an acetyl group is added onto a molecule. During phosphorylation, a phosphoryl group is added onto the molecule. In adenylation, an adenyl group is added onto the molecule. These processes are common methods for antibiotic inactivation. The modifications that these processes and the beta-lactamase inhibitors cause on the antibiotics are an efficient way for the cell to resist the effects of the antibiotic molecules.

1.9 Slowing the Rate of Resistance

 There are numerous ways that bacterial cells can become resistant to antibiotics and that is why the rate of resistance is so high and rising still. That is why it is important that we should be more aware of how we are using antibiotics and that we are doing what we can to make sure that we can slow down the rate of resistance. WHO has created a global action plan that provide us with guidelines for ways that we can slow the rate of resistance. The five main goals of this plan are to:

1. Increase awareness of antibiotic resistance

2. Strengthen knowledge through research

3. Reduce incidence of infection

4. Optimize the use of antimicrobial agents

5. Encourage investment into research for new medicines, vaccines, and diagnostic tools

1.10 Conclusion

 Antibiotics can be used for multiple situations ranging from treating infections in humans to growth promotion in animals. Since they are used so often, it is not surprising that bacterial cells are mutating to become resistant to them. There are many different ways that bacterial cells can be resistant to antibiotic molecules but there are just as many ways that bacterial cells can be susceptible to them. It is important that we spread awareness of resistance because if the rate continues to rise like it is then antibiotic resistant infections will become more likely. As the bacteria become more resistant, we begin to lack the correct antibiotics to be able to treat the infections. It is important that research continues to be conducted to find new antibiotics and that we take care to make sure that antibiotics are taken correctly and are not over prescribed.

VOCAB:

1. Antibiotic
2. Golden Age of Antibiotics
3. Anne Miller
4. Cocoanut Grove Fire
5. Antibiotic Resistance
6. Growth Promotion
7. Innate Resistance
8. Acquired Resistance
9. Biofilm
10. Persister Cells
11. Horizontal Gene Transfer
12. Vertical Transmission
13. Gram Positive
14. Gram Negative
15. Commensal Bacteria
16. Pathogenic Bacteria
17. Opportunistic Pathogens
18. ESKAPE Pathogens
19. Efflux Pumps
20. Specific Efflux Pumps
21. General Efflux Pumps
22. Proton Motive Force
23. ATP
24. MATE Pumps
25. MFS Pumps
26. SMR Pumps
27. RND Pumps
28. ABC Superfamily Pumps
29. Porin
30. Target Modification
31. Beta-lactam
32. Beta-lactamase
33. Beta-lactamase Inhibitors
34. Combination Therapy
35. Antibiotic Inactivation

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