Chapter 34: Antibiotics- An Overview

In this chapter we will discuss antibiotics, types of antibiotics, how they work, and how bacteria resist the action of antibiotics.

History of Antibiotics: Discovery

What are antibiotics? A good antibiotic must perform the following functions: must kill or inhibit bacterial growth, must not harm the host, must persist in humans long enough to have an effect with limited side effects, and must be effectively manufactured. There are medicines out there that do kill bacteria, but in turn also kill us. This would be an example of a bad antibiotic.

So how did we get to the antibiotic age, and what was before antibiotics? In ancient Rome, a man by the name of Galen, in 150 A.D., came up with the "Good Humors". "Good Humor" was saying that our bodies need to be in alignment with the four bodily fluids to be well. The four bodily fluids are: blood, yellow bile, black bile, and phlegm. If one of the four humors were off, an individual would become sick. Physicians would then use any means necessary to fix you; bloodletting, leeches, or other means to try and get your four humors back to balance. A little later in history, in the time of the Bible, people noticed that when one was in contact with another person who was sick, the individual would get sick as well. This is how the Asthma Theory was developed. In Greek, asthma means pollution, or foul air. In the 17$^{th}$ Century, Antonie Van Leeuwenhoek saw microbes for the first time, which lead to the further development of the investigation of medical necessity. The Good Humor theory might not be the cause of diseases, this giving way to another important discovery; vaccines. Edward Jenner formed the correlation between milk maids that were not getting sick from small pox. He thought it might have something to do with what they were exposed to with the cows. Jenner would inoculate them, thus coming up with the first vaccine. (Note vaca means cow). Robert Koch developed the germ theory in 1890. He formed postulates that a particular disease was caused by a particular microbe. He would then go on to isolate the microbe from the diseased patients. Next, he would take the isolated microbe and grow it on a plate in the lab, adding certain reactants to see what the microbe was. After this, he would give it to a healthy animal to see what happened. He then took the diseased host and isolated the microbe. This is the beginning to modern medicine and treatment as we know it. This had its fallbacks because some patients were asymptomatic, meaning they had no symptoms. However, still at this time, the four humors were still in effect. It would not be until much later, in the mid-1900's, that the new era of medicine and antibiotics would give way.

Antibiotic age mid 1900s

Paul Ehrlich was known for looking at stained cells under a microscope. Some cells were stained with the dye, and others were not. There was differential staining, which would help distinguish between different types of blood cells, which led to the ability to diagnose blood diseases. This would be the world's 1$^{st}$ chemical therapy. In 1891, methalyn blue was used to stain cells. This was used to treat malaria, although it was not the best therapy. It turned the whites of the eyes blue and the urine green. African sleeping sickness is a unicellular eukaryote much like malaria. However, now we are dealing with trypanosomes, which are much bigger than bacteria and much easier to see. They will now look for a

molecule with a particular shape, and a protein that will have a predicted activity. This leads the way to Salvarsan, which in 1910, was the most widely prescribed drug in the world. In 1912, they would improve upon this drug. This was called Neosalvarsan and had improved water solubility and treated diseases caused by microbes. In 1932, a German by the name of Gerhard, who was working for another company, was doing the same thing as Ehrlich. He was working on streptococcal diseases. He found if he gave mice the prontosil, and they were infected with a streptococcal disease, they would be cured. The scientists took the trials from animals and started testing these on humans. 1935 Prontosil was released. This drug works against spiral meningitis, pneumococcal, gonorrhea, and S. auerousis. This is the first antibacterial sulfa drug. So how exactly does this wonder drug Prontosil work? We will look at the chemical aspect of the drug. The side chains of the active part of the molecule, sulfonimine, is doing all the work. There are two rings, double bonded with nitrogen. On one ring, there is NH2 and asodye, and on the other side of the double bond, there is the sulfamylaidide, which is where the chemical bonds can be broken and distributed around. At this point, if one had strep throat, they would be given the sulfamide.

First antibiotic discovery: Penicillin

Alexander Fleming was the person who received credit for discovering penicillin. He had left a petri dish unattended which he had swabbed with He left for vacation and on the petri dish were staphylococci. Around the staphylococci, the bacteria died. The mold secreted something, so it stopped growing around the mold. He later identified as penicillin. It was noted the secretions are active against growth and harmless to animal cells. Unfortunately Fleming was unable to purify penicillin, he can not isolate it out and can not get enough to do anything with. Several people will take it from this stage to the drug that is widely available and know today. The Dunn School Team in Oxford was who will build an apparatus that can isolate and optimize growth medium on a plate. They developed a potency test and improved extraction for penicillin. In 1940, the group will now start testing this on mice and it does not harm the mice. Next, they will intentionally make the mice sick they will infect a disease into the mice and treat them. It works the first true antibiotic is formed the effectiveness is 1 part per million and 20 times more potent than prontosil. However with the war efforts going on they need money to get the drug out there. The group turns their efforts to America for this endeavor. A lab in Peoria, Illinois will be the back drop of the production of penicillin. Northern Lab will help with growing the aerobically penicillium, which needs a lot of surface area. They soon will figure out a way to extract using salt organic solvents to help purify the penicillin. They will find a hyper mold to help speed up the process and they will improve the growth and production methods and use a different medium and lastly, this will in turn scale up the entire process. They notice however that penicillin does not work against all diseases. Selman Waksman and Albert Schatz would like to work on mycobacterium tuberculosis the organism itself, their lab is sponsored by Merck under agreement that they will provide money for lab if they are able to discover the Merck gets rights and to manufacture them. They are specialist in actinomycin found in soil, they are famous for producing antibiotics, even better than penicillin. Soon they will start scanning and screening for different actinomycetes, looking for antibiotics to test. When they did they will look for any antibacterial component and if there are they will start the process to see if it kills bacteria and then not harming the host if there are none of these components they move on to the next one. When looking at the antibiotic they can tell if it is a broad spectrum meaning it will kill many bacteria, or a narrow antibiotic meaning it kills one or so. Also note there is minimal inhibitory

concentration of an antibiotic, this is the lowest concentration of an antibiotic that can stop bacteria from growing.

How antibiotics work

Animals and plants have a cell wall if the cell wall has a thick cell wall, this is Gram positive, in regards to antibiotics this is harder to kill the bacteria typically because of outer membrane the serves as a barrier to antibiotics coming into the cell. The peptidoglycan cell wall might think it acts as some kind of barrier it does not. The cell wall is like a chin link fence to water. You can pour a bucket of water right thru the fence. The peptidoglycan cell wall is full of holes whereas the outer membrane is not full of holes. Its tight, does not let antibiotics through. All the building blocks of the cell are located in the cell wall, DNA, RNA, protein, glucose. Doctors are normally more worried about Gram negative than Gram positive because harder to treat typically more resistance to antibiotics. So, bacteria cells are under an extreme amount of pressure known as turgor pressure. When this pressure builds up enough, they burst open and kills the cells. For example, penicillin prevents the cell wall from being able to enlarge and cell wall will eventually weaken causing the cell to burst. Because the cell wall also defines the size and the shape of cell unless the peptide glycan grows which means the cell has to grow while the cell is under pressure. If it wants new material must make space in the cell, which means has to break bonds, however if it breaks to many bonds BOOM the cell will burst killing the bacteria. So one way that an antibiotic can work is by limiting the cell which will cause it to die. If anything happens to the cell wall to synthesize it will die because it can not grow.

Both animal cells and bacterial cell have an essential role in bacterial growth and life. Remember we talked about the cell envelope and how it differs between Gram positive and Gram negative cells, but both have a peptidoglycan cell wall. This is one giant macromolecule. Antibiotics can target this or the precursors. What do cells have a lot of? Glucose. Now if we are targeting glucose we might be building up the precursor and getting it where it needs to be outside of the cell membrane. In manipulating these we have the power to essentially kill the cell by taking away the part of cell wall, which is needed for growth, again if the cell cant grow it will die.

Types of antibiotics: the most common and their targets

How do we classify antibiotics? Antibiotics are classified by what they target there are broad-, narrow-, and extended- antibiotics. Broad Penicillin the go to for antibiotics because it a narrow spectrum antibiotics and effects the Gram positive bacteria. A penicillin structure has beta lactam rings. The beta lactam was brought up before in this chapter as a naturally occurring square ring. All beta lactam antibiotics share this ring. There are 5 classes of beta lactam antibiotics. Penicillin is a class. You can normally tell the penicillin group because it normally always ends in -ilin. Examples are Amoxicillin, Methicillin, Epicillin, Carbenicillin, Piperacillin, Peculium. The second class of antibiotics in the beta lactams are Cephas porins, this is normally given to people who are allergic to penicillin they are named cephalexin, ceftriaxone, cefuroxime, cefazolin, cefadroxil, cefixime, nitrocefin. Next in this group are the carbapenems, imipenem, meropenem, relebactam all of these are carbapenems. Then are monobactams these include azactum, aztreonam, and cayston. This antibiotic targets the bacterial processes. This being the most common.

Other antibiotics focuses on protein synthesis. In bacteria we have the ribosomal sub units we are a 30s, which is smaller and has rRna-r just stands for ribosomal and we have a bigger unit 50s with rRna in it. If you think of a marking gun there is one side that has what is to be printed and a tape in the middle and then on the other end the gun is holding it together, think of this as the bacteria. Now we have messenger RNA in the middle, the messenger RNA tells us what we are going to be making. The tape will be the actual mRNA in this scenario, and we have the building blocks that connect which are tRNA, t we can think of making or letting the cell know what we are making. Now on both sides of a marking gun they have to match this is like an anticodon. An anticodon must match with a site. Let us call the site EPA. So, we have the tape going thru like an A for activation and the P for processing and E for Exit. Now the have to make the amino acid just like any amino acid in our bodies they make proteins, so we have initiation factors and release factors and making factors=the EPA. Some antibiotics will target the beginning of the cell, so the cell cannot make or complete the bacteria. Other antibiotics target the P site, which if you think of processing is actually called the elongation site, on the elongation site if the bacteria cant get out the cell will die, or last the exit and again if the cell can not make it out of this site it will die.

Next there are bacterial translation inhibitors, this competes with the initiator tRNA at the P and E sites. kasugamycin, this is not a clinical antibiotic, just experimental. This would bind at the start codon the kasugamycin would compete with the initiator to bind at the P=again think processing site and would bind at this same site.

Tetracyclines.

Oxytetracycline, chlortetracycline and second generation doxycycline. These antibiotics block Elongation, the process they actually interfere with is the accommodation process. A=activation site disrupts the codon and anticodon remember it has to link like the marking gun. The bacteria will never get underway because it can never match, therefore never leaving.

Aminoglycosides

This antibiotic also works against elongation but in a different way. These antibiotics include Kanamycin, gentamycin, tobramycin and the newer generation Amikacin and Neomycin. So how it works is more tRNA tries to come in the A site however the aminoglycosides bind at the same place. This stabilizes close matches which would be wrong and not make a bacterium. The wrong amino acid which is a building block and therefore you cannot make your bacteria.

Oxazolidinones

These also block the elongation process, however you guessed it, a different step. Peptidyl transfer center. So once you have an amino acid the next step is the peptidyl transfer. This antibiotic blocks the peptide transfer from occurring.

Macrolides

These antibiotics are protein synthesis inhibitors and block the exit, hindering the peptide exit. The bacteria can only get to six or eight amino acids and cannot make it out of the cell. The Z pack as we know it, Azithromycin is very commonly prescribed helps with strep throat, ear infections, and lots of childhood sickness. Azithromycin and clarithromycin blocks the exit tunnels. The amino acids can only

get to six or eight amino acids. Other macrolides cause shorter amino acids, which in turn leads to no bacteria. In this category there is also, chloraphenitnitro benze or chloramphenicol these two antibiotics are very cheap to make pretty simple and powerful antibiotic not used in the US very often because there is a small risk of aplastic anemia or cancer. This antibiotic binds on the peptidyl center and blocks peptidyl transfer when needs to add next amino acid it cannot do it. Syneroid, here we have two different products that will be taken together in a 7;3 ration dalfopristin: quinupristin B the dalfopristin is for the streptogramin A and quinupristin is for streptogramin B these two are working synergistically they work better together than apart. These antibiotics are mainly used for vancomycin resistance. Each antibiotic on its own is bacteriostatic stops bacteria from growing, but together bactericidal, which means causes death of the bacteria. Quinupristin blocks exit site, while dalfopristin blocks the peptidyl transfer. They are used to treat staphylococcal infections, VR Enterococcus faecium, and streptogramins. Clindamycin was discovered in Lincoln, Nebraska and is a derivative of Lincomycin and also binds to the exit tunnel. Clinical uses for this antibiotic include swimmers ear, bone/joint infection, strep, MRSA, pelvic inflammatory disease. It must be noted also if you use this for intestinal infection it can kill the norma flora in the microbiome causing c. diff. The three antibiotics that work on the exit tunnel are macrolides, quinupristin, and clindamycin. Kirromyocin, this pre-clinical drug is to target the elongation factor thermos unstable is another elongation factor, where it binds and overlaps where the tRNA binds. Erythromycin and clarithromycin bind to the site of the exit tunnel.

Topoisomerase Inhibitors.

We know that our DNA is double supercoiled, bacteria work to keep their DNA negatively coiled state. This is easy to explain like our DNA is like a staircase that is circular and is right-handed meaning it goes left to right as we go up. This allows cells to easily unwind, DNA is already unwound, and energetically easier i.e.- easier replication and transcription. Unwinding thru a series of proteins the supercoiling can introduce a topo isomer which are the same chemicals but a different structure. When the DNA is unwound there becomes physical strain on the structure. When we unwind the helix that tension has to go somewhere, it goes into the left handed super coils. Unwinding through a series of proteins DNA supercoiling can introduce a topoisomer. Topoisomer has the same chemicals different structure. DNA gyrase is an enzyme that catalyzes the energy dependent. The gyrase has 4 parts 2As and 2Bs these type 2 toperosomerase. They use ATP to make supercoil, no need to spin energy, add negative super coils and can relieve positive super coils without needing ATP. Negative (right-handed) supercoiling is the natural stae of bacterial DNA due to gyrase activity, which previously stated makes it easier to unwind. Positive or lefthanded supercoiling occurs when DNA is unwound, as during replication and relaxed by gyrase.

The other is to unlink or decapitate circular DNA molecules after replication. You have two interlocking molecules and make them two separate molecules. Again, we know DNA needs to keep their chromosomes negatively coiled. So, after replication they successfully divide. They cut and rejoin the strands. Topoisomerase break, pass, and rejoin DNA helixes ligate. So gyrase binds to DNA molecule and then breaks DNA molecule, the subunits holding on the ends the other one would passes thru space created, and then it changes the shape. During this intermediate period the DNA is cut and covalently bound to the gyrase A until it is restored. This makes for a great antibiotic target, the mechanics of the antibiotic were discovered way after the antibiotics themselves. Now we will talk about the Quinolones and Fluoroquinolones these two antibiotics target DNA topoisomerases. Quinolones synthetic and discovered accidently, not a great antibiotic very narrow spectrum. Fluroquinolones, ciprofloxacin,

norfloxacin, levofloxacin these all target gyrases A subunit. The 3rd generation is Moxifloxacin, and it inhibits both DNA gyrase and tropo. 4th generation is Delafloxacin discovered in 2017, this antibiotic will bind to DNA gyrase A and stabilize the intermediate structure when the DNA helix is broken so DNA breaks and then causes cell death. Delafloxacin is used for infections, skin, respiratory, UTI, pelvic inflammatory diseases, diarrhea. When the bacteria cell is broken attaches itself to gyrase so cannot finish the job, which means cant put ends the back together. All the fluoroquines target gyrase A.

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