Title: Bioinformatics of Antibiotic Resistant Bacteria: New Delhi Metallo- β -Lactamase

By

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Abstract:

The goal of my study was to find out how antibiotics work and how bacteria are escaping antibiotics by using the bioinformatics approach to understand the process. Antibiotic resistant bacteria that are difficult to treat are becoming increasingly common and are causing a global health crisis. These types of deadly bacteria are known as superbugs. Every year in the United States, at least two million people become infected with bacteria that are resistant to antibiotics and at least 23,000 people die each year as a direct result of these infections. In addition, the current death toll is 700,000 worldwide due to antibiotic resistant bacteria. However, it is estimated that by the year 2050, it will balloon to ten million deaths every year. To give a better perspective, that is more than the 8.2 million per year who currently die of cancer and the 1.5 million who die of diabetes, combined. This global crisis can be prevented if action is taken soon. The tools used in my research were the Protein Data Bank, Uniprot, Drugbank, and Chimera. All of these resources were used to gather useful information on the resistant bacteria and the antibiotics to show and understand how they work.

Introduction:

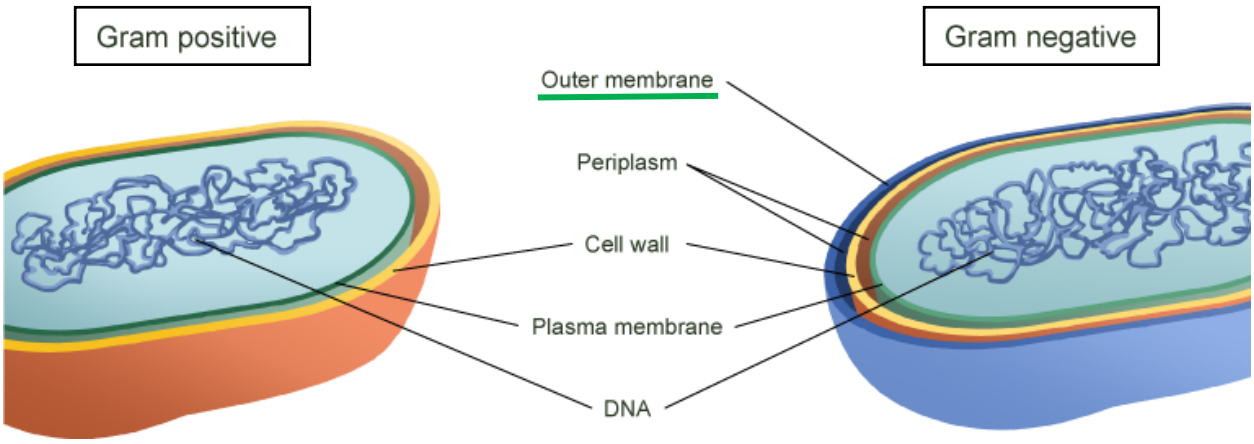
Bioinformatics is the science of analyzing macromolecules by using certain techniques to observe, understand, and gather information of the molecules. Bioinformatics includes information of the macromolecule structure, DNA sequence, and a protein's structure and its purpose. Computer science and mathematics are used to compute the biological problems.

When a person develops a bacterial infection, the doctor will prescribe to them an antibiotic. Moreover, there are well over one hundred different kinds of antibiotics that treat different kinds of sicknesses. However, these superbugs are strains of bacteria that have evolved after coming into contact with the antibiotics. These bacteria go through a process of becoming resistant to many of the antibiotics and then they begin to multiply. The scary thing is that these superbugs can even develop during the course of a person taking the antibiotics. The type of bacteria that are superbugs follow the acronym ESKAPE:

E: Enterococcus faecium
S: Staphylococcus aureus
K: Klebsiella pneumoniae
A: Acinetobacter
P: Pseudomonas aeruginosa
E: Enterobacter

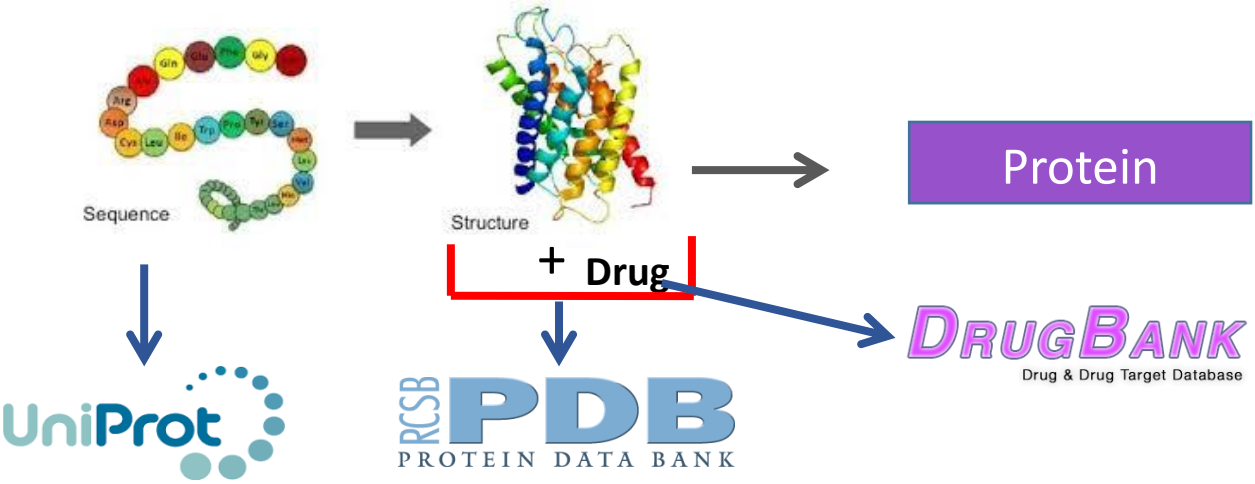
Generally, there are two types of bacteria. There are gram negative bacteria and there are gram positive bacteria. They both have similar structures that make up the cell wall but one of

the difference is that gram negative has an extra membrane layer on the outside that gives it further protection which makes it harder for the antibiotic to do its job of destroying the bacteria. Therefore, it is mostly these gram negative bacteria that become the more resistant out of the two.



(figure 1)

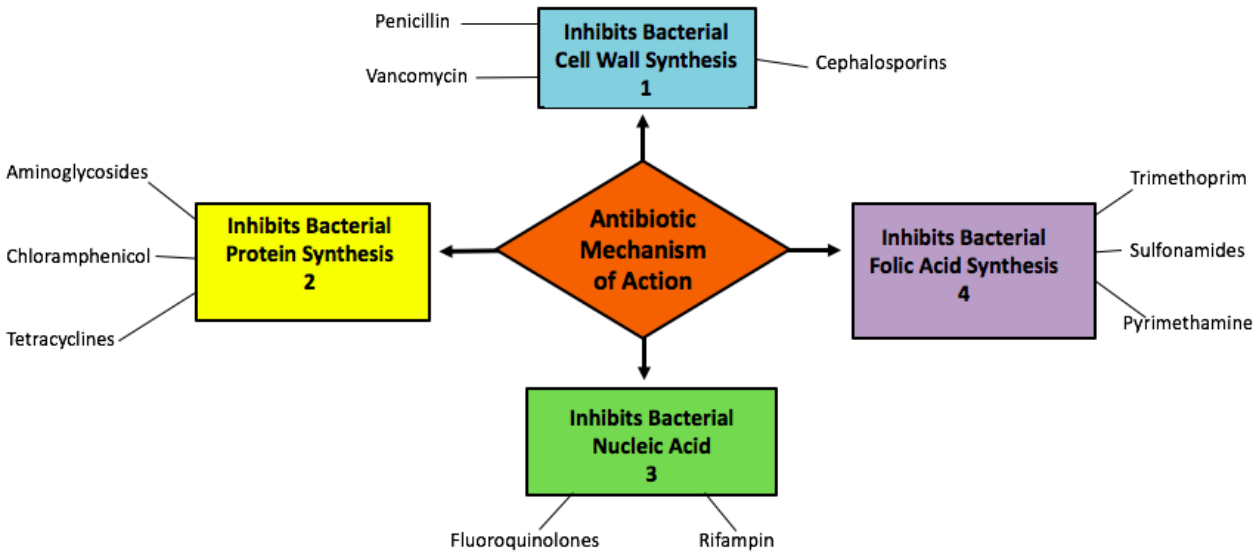
"Form follows function" which means that a structure within an organism is related to its purpose. Proteins fold in three dimensions and its structures are formed in direct correlation to what they are meant to do. The three main websites that I mainly used for my research are Uniprot.org, Rcsb.org (the Protein Data Bank), and Drubank.ca. In order to gather information on the bacteria and the antibiotics, I followed certain steps to achieve this. First, to get the primary structure of the protein I retrieved it from the Uniprot database which is a central repository of protein sequence data. Next, to find the three dimensional protein structures I went to the Protein Data Bank which is a database for three dimensional structural data of large biological molecules, such as proteins and nucleic acids. And then last, I obtained the drug information from the Drug Bank which is bioinformatics and cheminformatics resource that combines detailed drug data with comprehensive drug target information.



(figure 2)

Experimental Section:

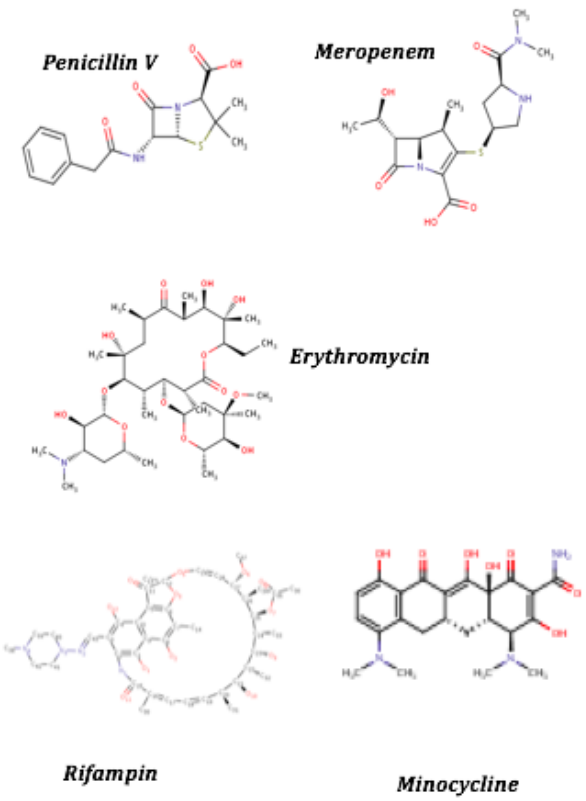
The following diagram shows the different ways and mechanisms that antibiotics attack and kill the bacteria along side with examples of different types of antibiotics listed that cause each mechanism.



(Figure 3)

The first mechanism is inhibition of cell wall synthesis. The antibiotics job is to stop the cell wall from producing. The second mechanism is the inhibition of protein synthesis. Without its proteins, the bacteria can't carry out vital functions, including asexual reproduction. The third mechanism is inhibition of nucleic acid synthesis. Nucleic acid is what makes up DNA, and without it the Bacteria cannot form. And the fourth mechanism is inhibition of folic acid. Folic acid is needed in order for bacteria to grow and without it the bacteria will die. All in all, certain antibiotics will target at different sites of bacteria.

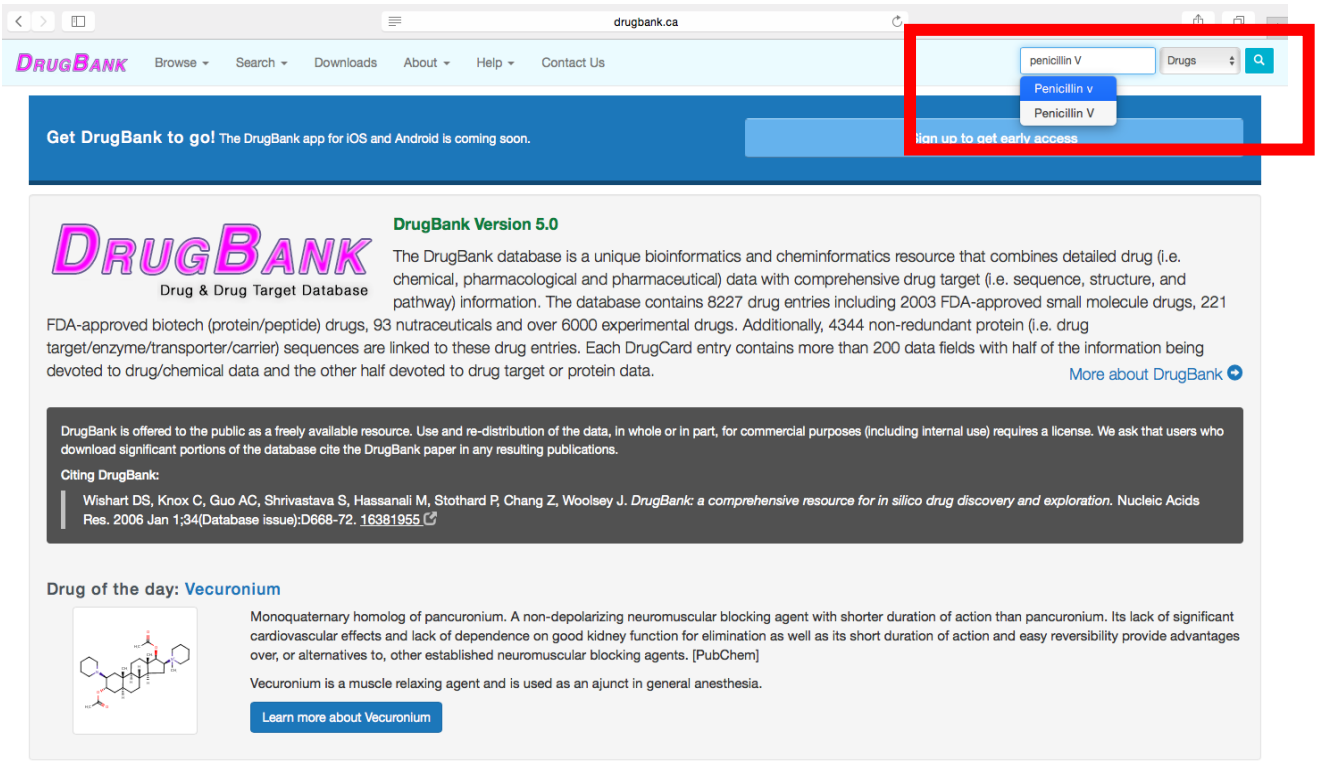
Shown below are some of the structures of the antibiotics which will be mentioned about later on this paper.



(Figure 4)

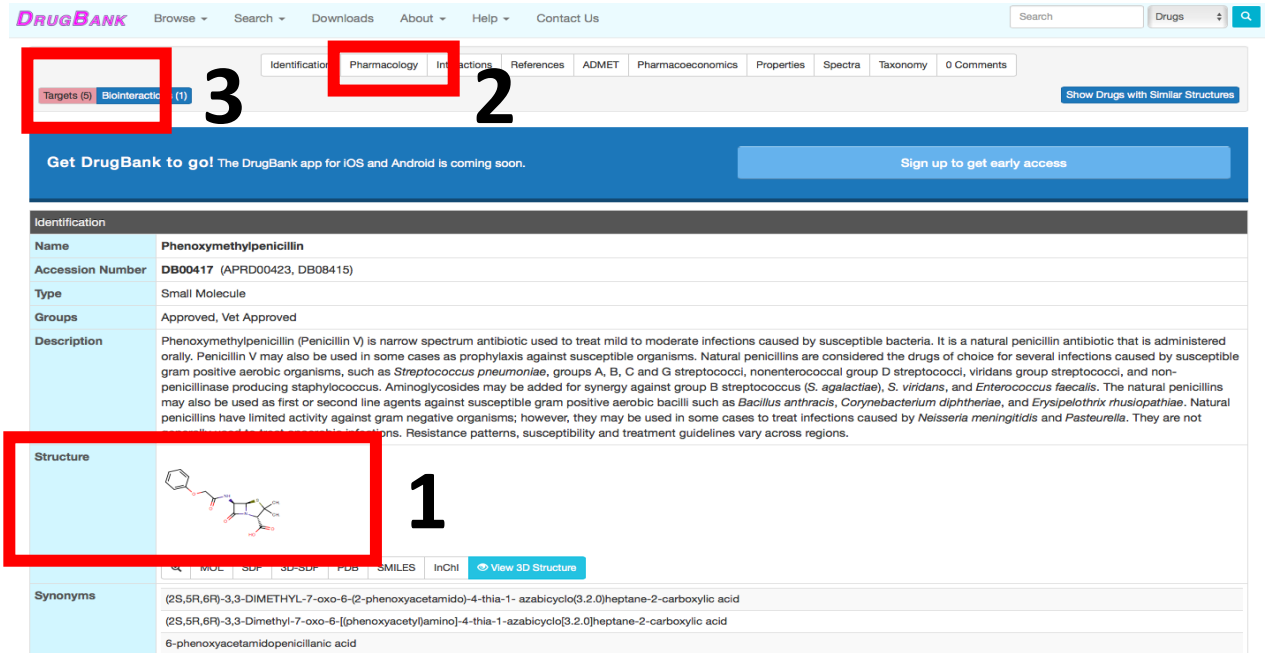
Now I'm going to tell you the first part of my research of how antibiotics work. Before, I showed you four ways, but now I will be focusing on just one of them. Someone I know told me of how they went to the doctor and were prescribed with Penicillin V due to a bacterial infection. Being curious of how the antibiotic kills the bacteria, I decided for demonstrative purposes to focus on this drug. I went through the same steps of going to the Drugbank to find information on Penicillin V, and then went to Uniprot, & then the Protein Data Bank.

First, by going to the Drug Bank I searched for penicillin V in the search box.



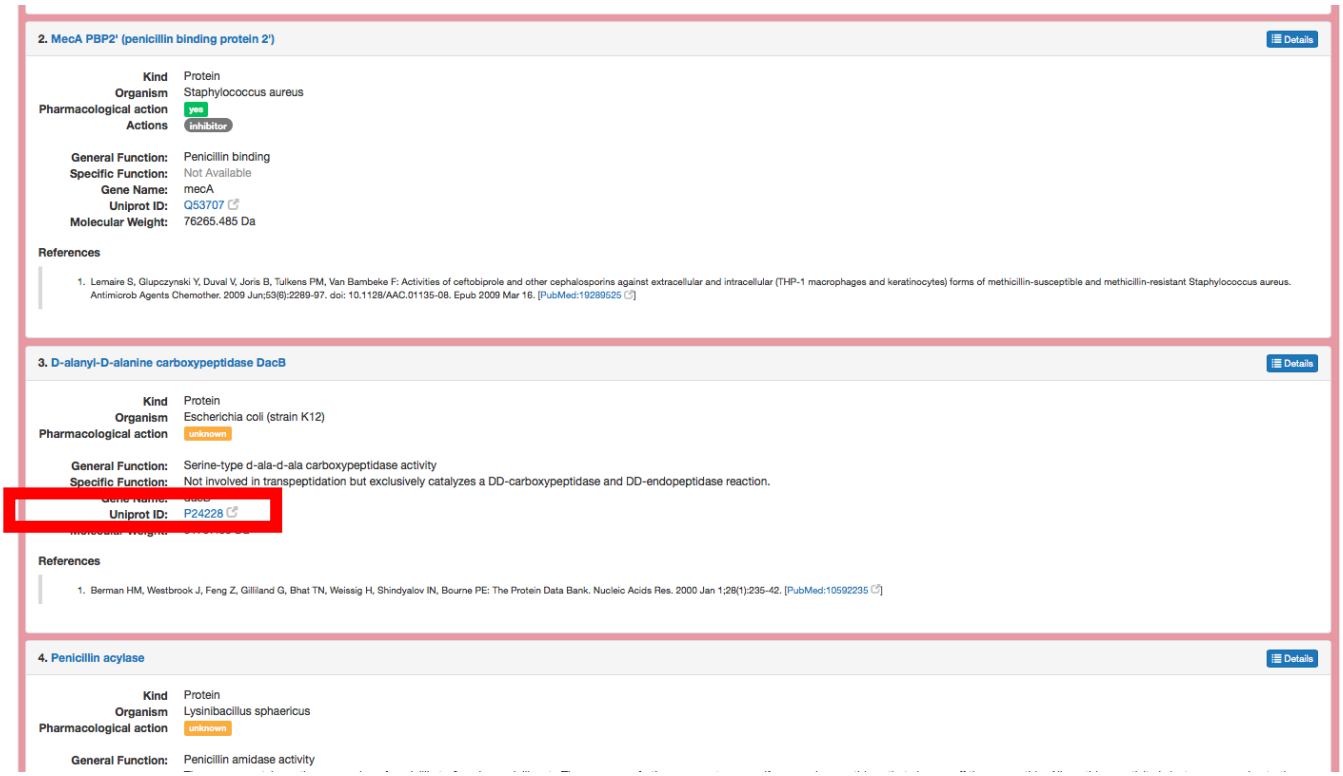
(Figure 5)

1. Next, I found the structure of the drug
2. When I clicked on pharmacology, that is where I found the mechanism of action, which is where it explains how the drug is interacting with the bacteria
3. Then when I clicked on targets, it brought me to the five different proteins that it binds to



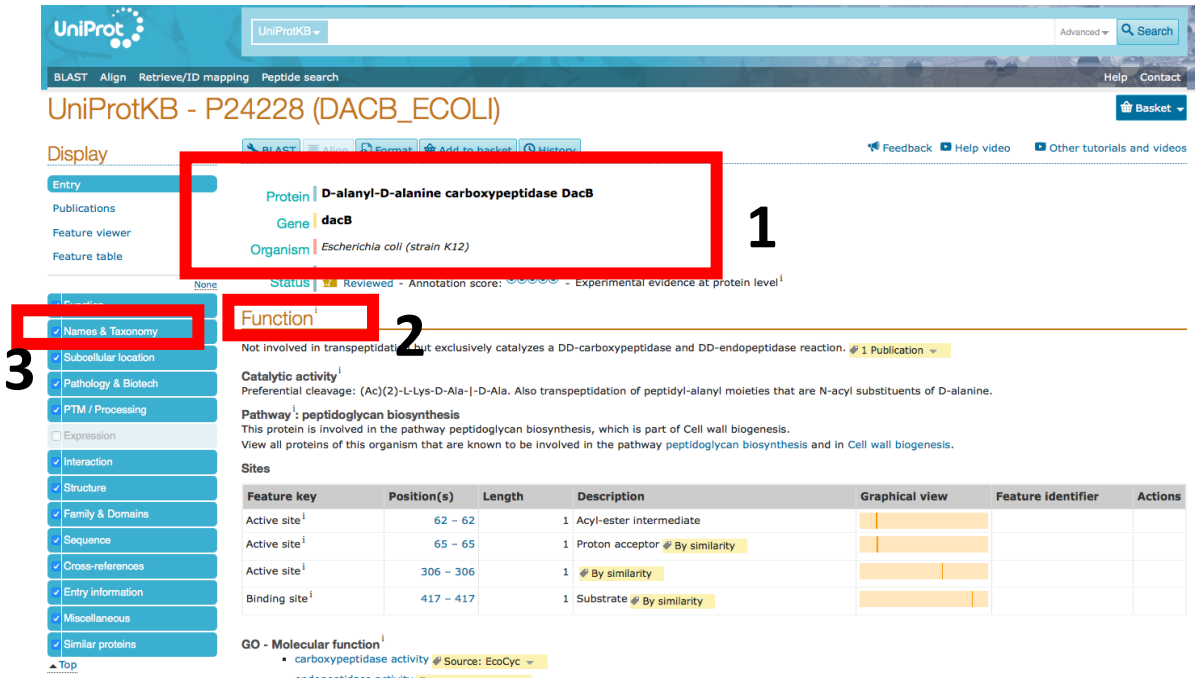
(Figure 6)

This is the list of five different proteins. Each protein has a Uniprot ID number that will bring you to the Uniprot website to get the sequence of that protein. The Drugbank provides a lot of valuable information but I focused on specific parts that I used for my research.



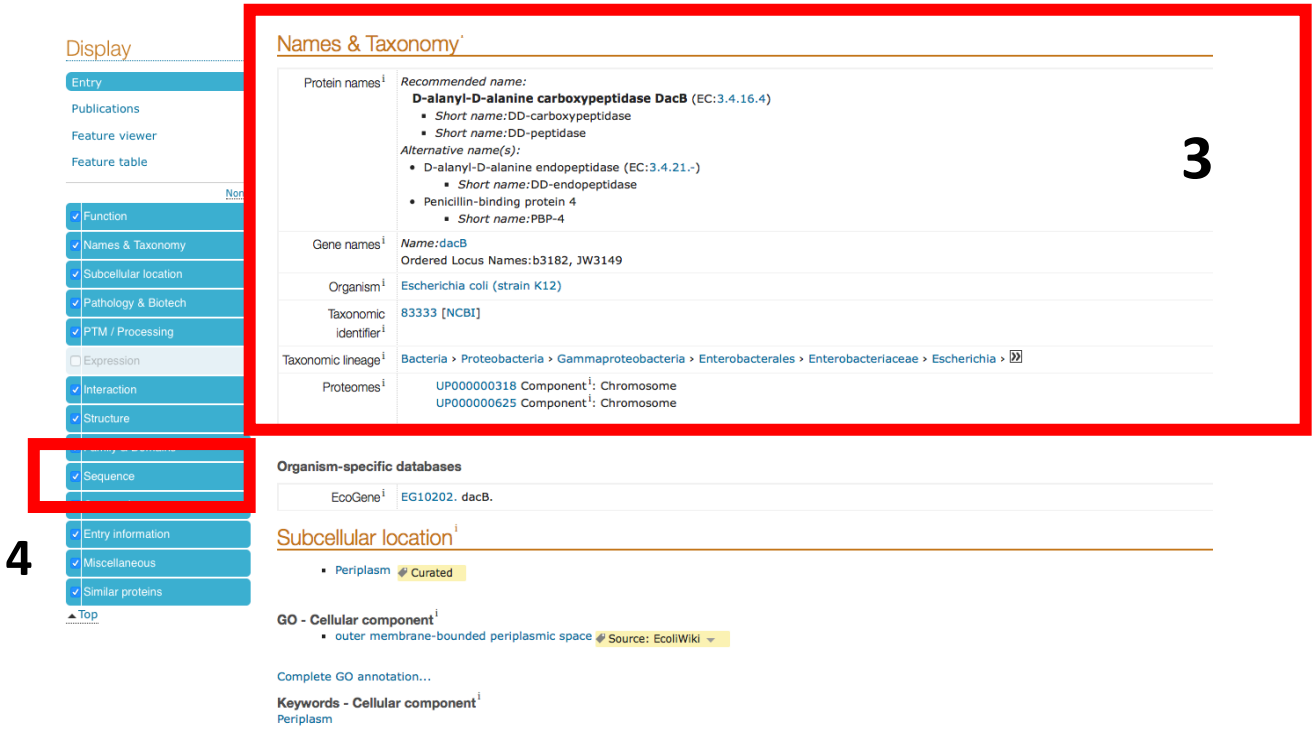
(Figure 7)

The next step is to go to the Uniprot website to get the sequence. By going back to the target sites from the Drugbank and clicking on the Uniprot ID number, it will bring you to the website and we will find valuable information on the protein.



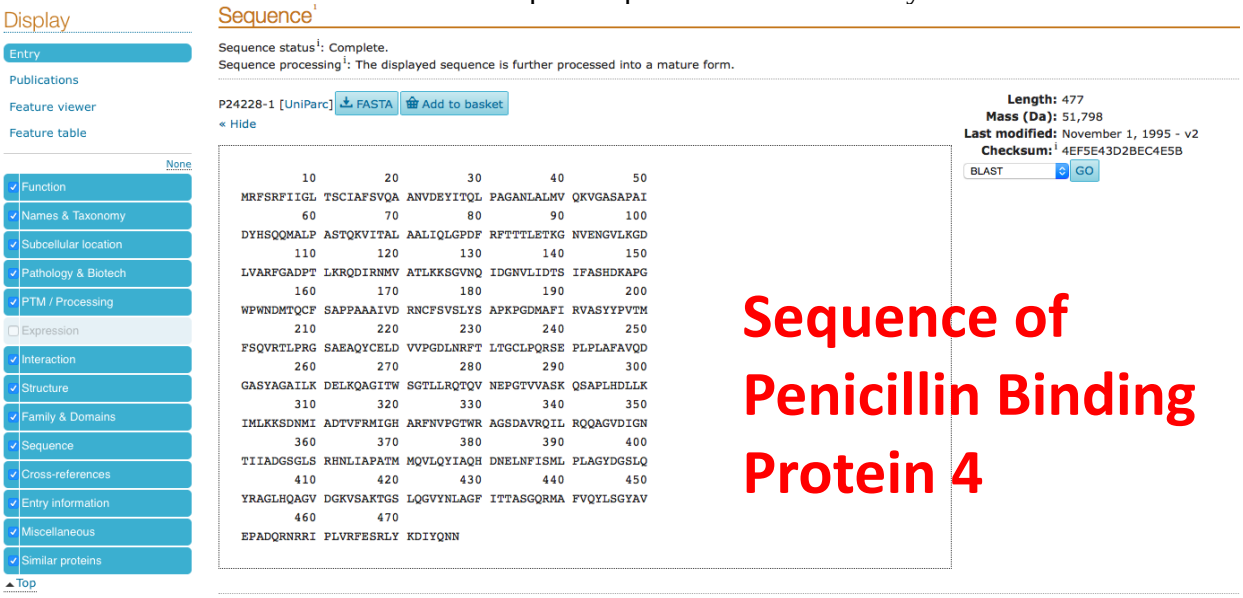
(Figure 8)

1. I found the protein name
2. The function of the protein is found. This protein is important for bacterial cell wall synthesis
3. Then I clicked on Names & Taxonomy



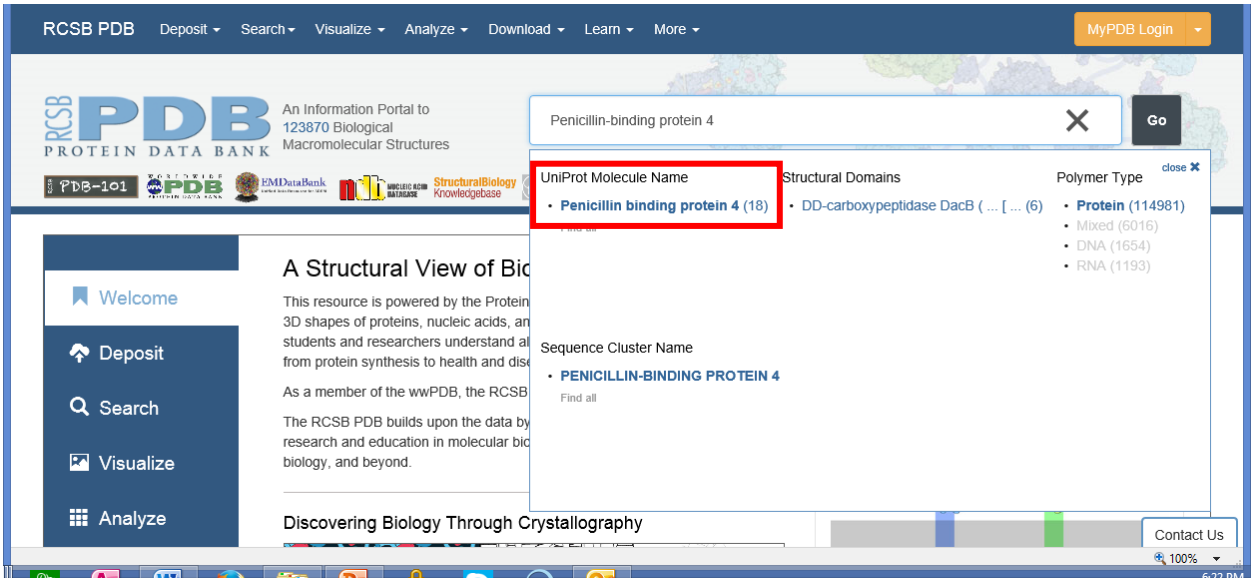
(Figure 9)

I found a list of synonyms for the same protein and I chose the easier and more common name that is used which is “Penicillin binding protein 4”. Also on the left hand side, by clicking on sequence tab, it brought me to the sequence of this protein. The Uniprot website provides a lot of valuable information but I focused on specific parts that I used for my research.



(Figure 10)

After finding the sequence of the protein, the next step is to actually find its structure. And that is done by going to the Protein Data Bank website. When going to the Protein Data Bank website, I typed in “Penicillin Binding Protein 4” and there were eighteen structures of this protein that appeared.

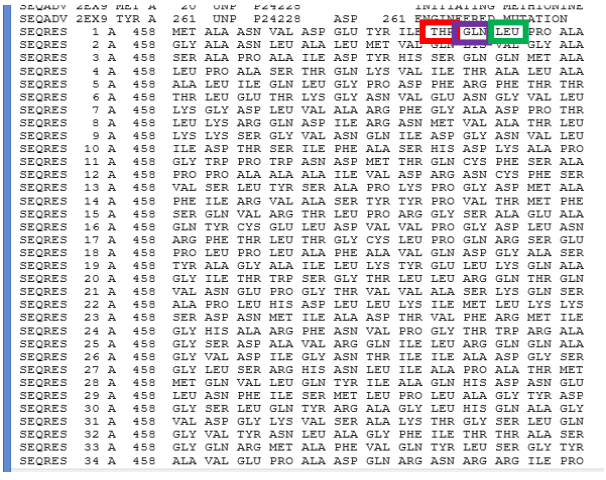


(Figure 11)

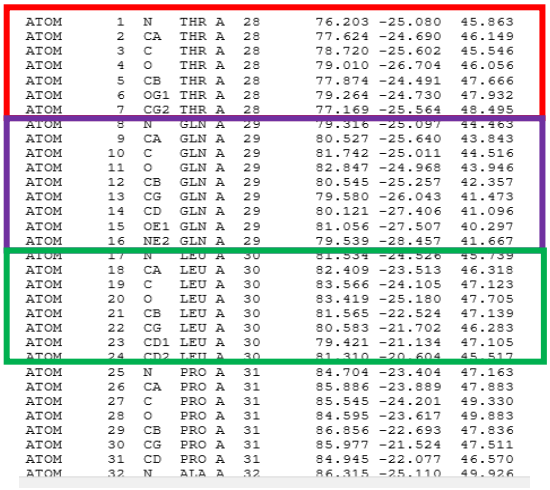
The entry that I chose was 2EX9 because it was complexed with “penicillin V” which is the antibiotic that we originally searched for in the Drug Bank.

(Figure 12)

After clicking on the entry of choice, it directed to the page that gives a structure summary of the whole entry. On this page, there is a lot of value information but I focused on specific parts that I used for my research. On this page the structure image, full protein name, and macromolecules, was found. To find further details on this entry, I clicked on the PDB file that is the encyclopedia of the three dimensional crystal structure of this protein. This file has a lot of important information, however, I focused on how the sequence translates to its structure. Next, I focused on how the sequence translates to its structure. I found the sequence of the protein which show all the amino acids that make up the protein for each residue and it breaks it down further to show its atoms.



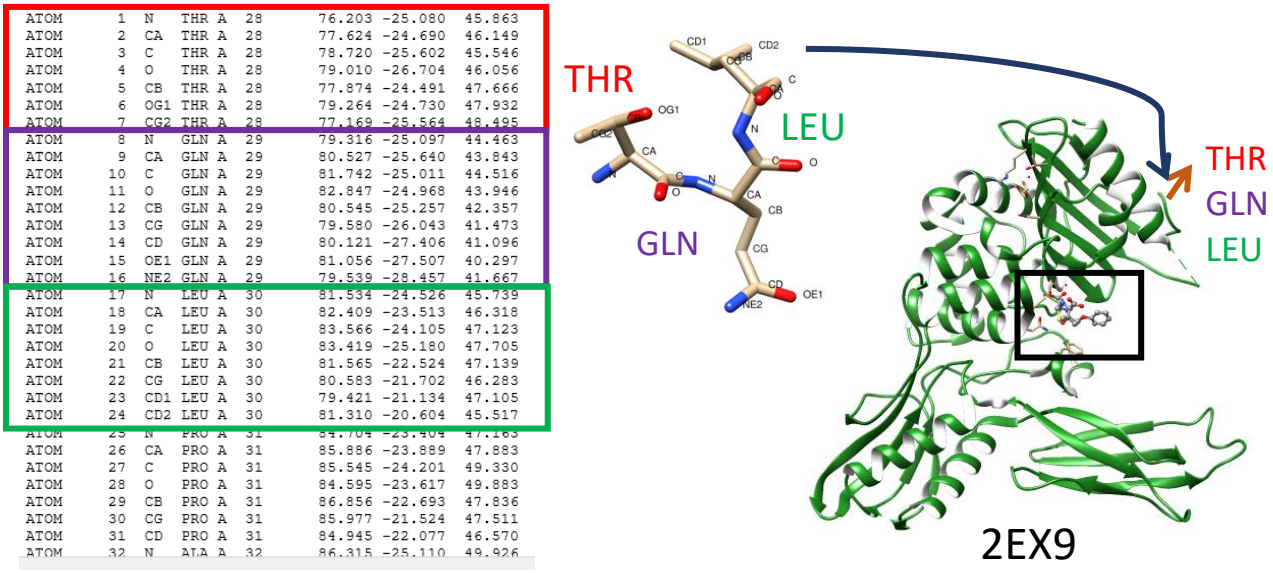
Protein sequence studied in this entry 2EX9



Protein coordinates in entry 2EX9 obtained by X-ray crystallography

(Figure 13)

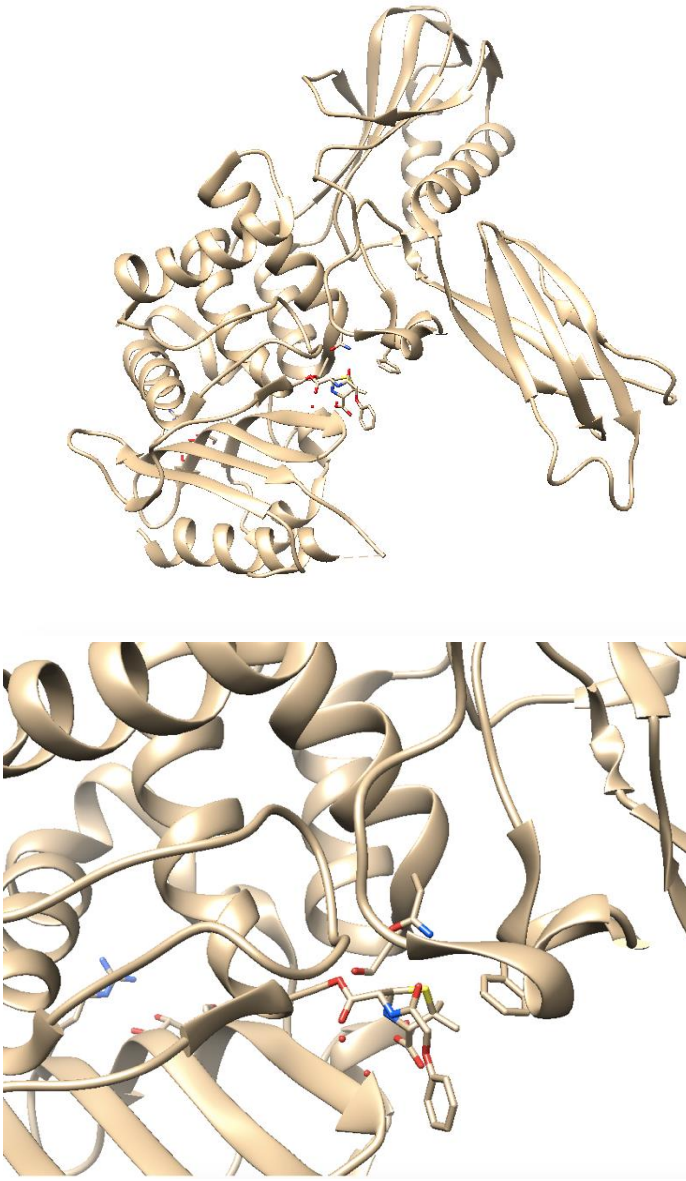
The sequence shows the first three visible amino acids and for each amino acid it shows the atoms that makes it up. For each atom there is an x,y,z coordinate which is the mathematical numbers of the three dimensional crystal structure of the protein. These coordinates are what translate into a three dimensional image.



(Figure 14)

Those x,y,z coordinates translate the atoms into this three dimensional picture (middle picture). The atoms makeup the first three amino acids. All of the atoms and their coordinates makeup the full three dimensional structure of the protein (right picture). However, by looking at this file it may be hard understanding exactly what is going on. To understand it better I pulled up this entry in a three dimensional viewer called Chimera. When translating these x,y,z coordinates of the atoms into a three dimensional picture one will be able to view it better.

First, I went to Chimera, and typed in the entry 2EX9 from the Protein Data Bank in the "fetch by id" box. The whole protein structure will appear with Penicillin V bound to it. In Chimera I learned how to use the tools and different features and how to modify the molecule.



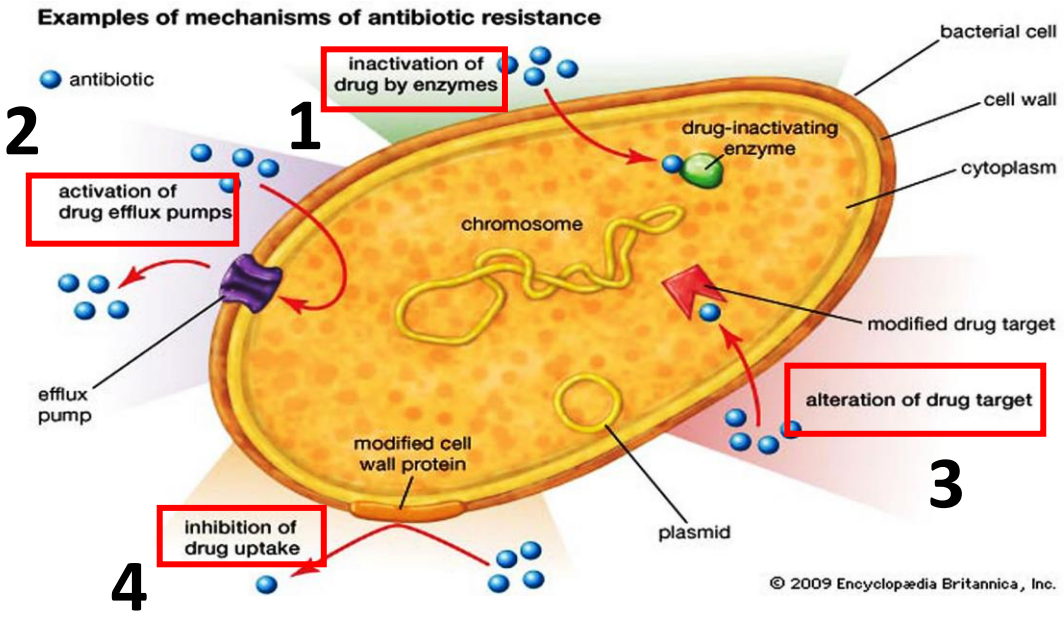
(Figure 15)

In this image it is shown that the penicillin beta lactam ring is broken. The Antibiotic is binding to the Serine 62 of the Penicillin-Binding Protein 4. This serine is what helps to make the cell wall but the antibiotic is now blocking serine 62 and therefore, the bacteria cannot make cell wall causing it die.

Results and Discussion:

Previously I explained the different ways how antibiotics work. Now I am going to go to the second part of my research and show the different ways of how bacteria escape antibiotics.

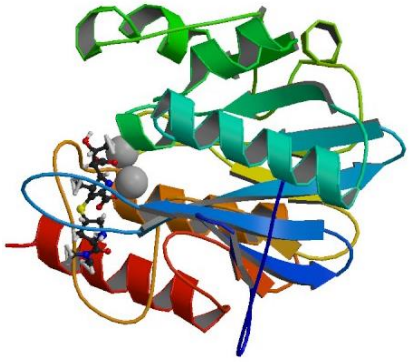
Shown in figure 15, is a bacterial cell and the blue circles symbolize the antibiotics molecules. There are many mechanisms of how these bacteria are escaping the antibiotics. The most common mechanism is inactivation of the drug by enzymes. The bacteria develop enzymes that are capable of inactivating the antibiotics. Another mechanism is efflux pump. The antibiotics go inside the bacterial cell and there are special molecular pumps that pump the antibiotics out of the bacteria cell. The next mechanism is that the bacteria goes and changes the drug target so that antibiotic cannot bind to drug target site and then the antibiotic becomes useless. For example, we can refer previously to the penicillin V binding to penicillin binding protein 4. The bacteria can go and change the structure of Penicillin Binding Protein 4 so that the antibiotic will not be able to bind to it. The last mechanism is where bacteria modifies its cell wall so that drugs cannot even penetrate and get inside. Due to limited time in my research, I focused and elaborated on the first two mechanisms only.



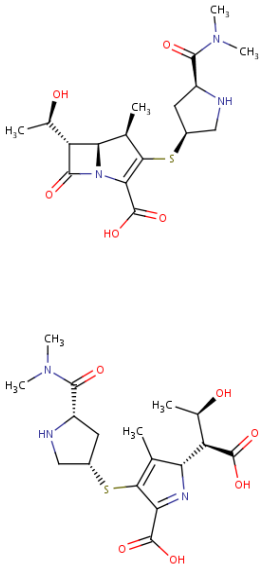
(Figure 16)

Next in my research I will discuss the mechanism of inactivation of drug by enzymes. NDM-1 is a deadly enzyme. By using this molecule, bacteria can escape most antibiotics and that is why it is called a superbug. One of the most dangerous strains arose in south Asia and recently caused a series of deadly infections in Los Angeles area medical centers. Most enzymes surround their substrates, so that they recognize only their specific target molecules. NDM-1, on the other hand, recognizes the key reactive portion of the antibiotics by using two zinc ions, but ignores the rest of the molecule. That is how it can disable nearly all β-lactam antibiotics. The entry 4EYL from the Protein Data Bank highlights how NDM-1 destroys the antibiotic, Meropenem.

Figure 17, shows the structure of NDM-1, the two zinc ions coming from the bacteria, and the antibiotic Meropenem.



(Figure 17)



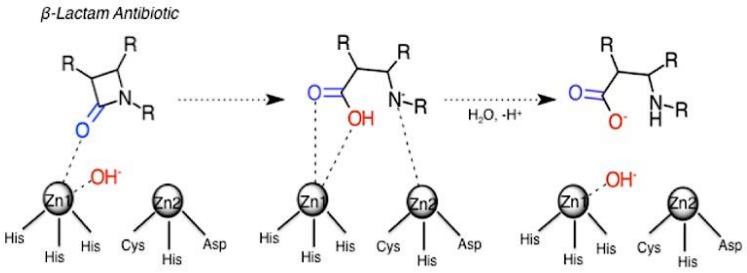


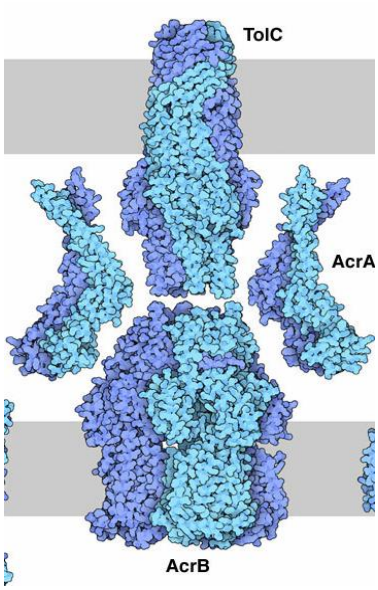
Figure 1. *β*-Lactam hydrolysis mediated by class-B1 metallo-*β*-lactamases.

(Figure 18)

Figure 18, shows the intact structure of the antibiotic compared to after NDM-1 has worked on it. The enzyme NDM-1 breaks the beta lactam ring of the antibiotic and makes its inactive by hydroxylating the beta lactam ring and changing its structure. The enzyme then becomes free again to inactivate the next antibiotic.

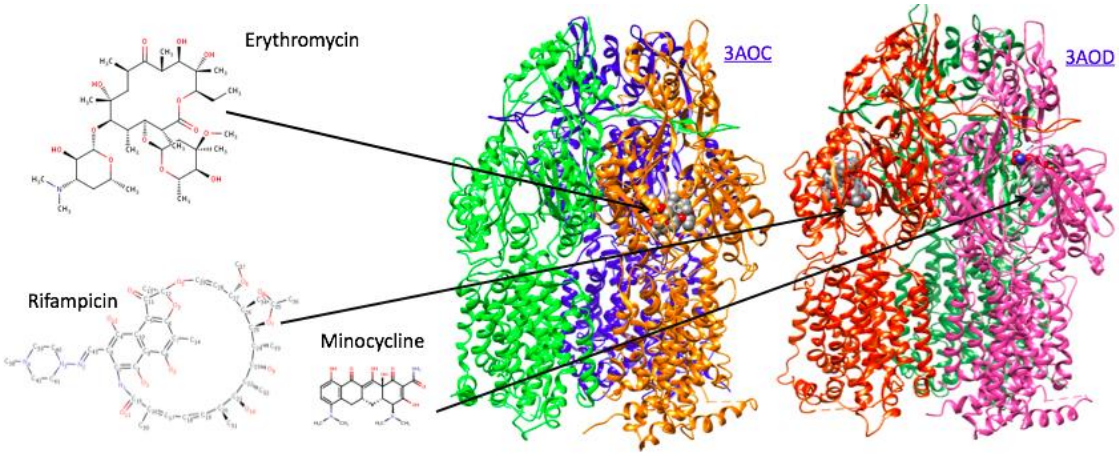
Next, in my research I am going to discuss the efflux pumps in the resistant bacteria. There are many different types of pumps but I will specifically be focusing on AcrB Transporter Pump. The AcrB transporter pumps drugs out of the inner membrane of *Escherichia coli* and into a tube formed by TolC, which directs the drugs all the way out through the outer membrane of the cell.

The protein AcrA is thought to form a ring that connects AcrB and TolC, linking the entire complex into a closed tube.



(Figure 19)

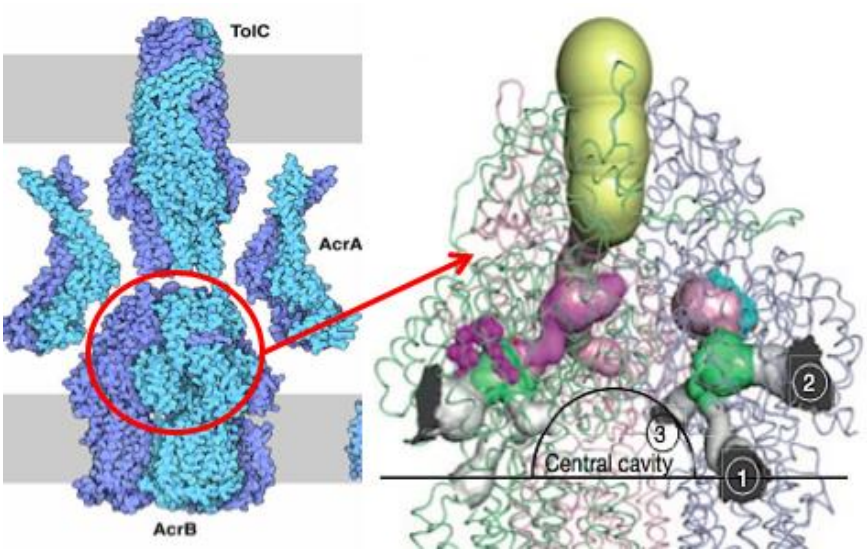
Next, in my research, I explored how AcrB pumps antibiotics out of bacterial cells. In Figure 20, these are two ArcB Protein structures from the Protein Data Bank entry 3AOC and 3AOD. These proteins form a tube by using three different chains colored in the three different colors. This protein is large and has many cavities that can bind many drugs in all different places at once. Erythromycin is bound to 3AOC and Rifampicin and Minocycline are bound to 3AOD.



(Figure 20)

This is a big protein with many cavities and because it has so many cavities it can accommodate many antibiotic molecules at once. The authors suggest that AcrB acts like an elevator, where several drug molecules board, the protein moves by a peristaltic movement and ejects the drug molecules. Then AcrB reverts to its former shape and gets boarded again by more

drugs. The authors hypothesize that the drugs are pushed from AcrB to TolC and out of the cell. That is probably how the drug goes from the inside to the outside of the bacterial cell (shown in figure 21).



(Figure 21)

The drugs are pumped through the green binding pocket and pink binding pocket and finally leaves through the yellow exit funnel.

Conclusion:

From bioinformatics, I learned extensively how to use Uniprot, Protein Data Bank, Drugbank, and Chimera. I was able to gather useful information for my research. The purpose was to show the importance and to bring awareness of bacteria resistance because antibiotic resistant bacteria is a major global crisis. NDM-1 is deadly enzyme. By using this molecule, bacteria can escape most antibiotics and that is why it is a superbug. NDM-1 recognizes the key reactive portion of the antibiotics using two zinc ions, but ignores the rest of the molecule. This allows it to disable nearly all β-lactam antibiotics. There are still a handful of strong antibiotics that can attack these antibiotic resistant bacteria during the time being. However, these superbugs are advancing quickly and scientists are currently trying to find a way to solve this problem before it gets out of hand.

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