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The Effects of Amikacin, Doxycycline, Erythromycin, Penicillin, and Sulfamethoxazole with Trimethoprim on Tylosin Resistant E. coli

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ABSTRACT

Antimicrobial resistance has been an issue since Alexander Fleming discovered Penicillin in 1928. In today's society it is considered a health crisis. Not only are there more resistant strains of bacteria, but there are bacteria that are becoming multidrug resistant. There are many reasons for the increase of antibiotic resistance, and at least four mechanisms bacteria can use to become resistant. They have found that bacteria can be resistant to a whole class of antimicrobials, or even many classes. Resistance not only affects humans but also the agricultural industry. It is important to discover the extent of resistance and how it can affect animals and humans. An *Escherichia coli* strain was isolated from a feed lot that showed resistance to Tylosin. Using the Agar Disk Diffusion Method, I tested this strain against Amikacin, Doxycycline, Erythromycin, Sulfamethoxazole with Trimethoprim, and Penicillin to see if resistance to Tylosin affected its resistance to other antimicrobials. I chose to use these antimicrobials because of the families, or classes, they were in. Erythromycin is a macrolide like Tylosin, but the other four antimicrobials come from different families with different mechanisms. I found that this strain of *E. coli* showed more resistance when compared to the control strain for Amikacin, Doxycycline, Erythromycin, and Penicillin, and less resistance to Sulfamethoxazole with Trimethoprim. However there were some problems with my Penicillin control which make the results unreliable.

Keywords: *Antibiotic Resistance, E. coli, Amikacin, Doxycycline, Erythromycin, Sulfamethoxazole with Trimethoprim, Tylosin.*

INTRODUCTION

Antibiotics (now more traditionally called antimicrobials) were discovered in the late 1920's. The first major discoveries were made by Alexander Fleming, penicillin, and Gerhard Domagk, sulfa (Turkoski, 2005). Just a few years after these discoveries were made, researchers found bacterial strains that were already becoming resistant to these new antimicrobials (Accelr8, 2007). For a long time medical professionals were able to keep resistance at bay by forming new (or new forms of old) drugs. For each resistant strain they formed a new antimicrobial. However in the past few decades resistance has been growing faster and scientists are having a hard time keeping up with new antimicrobials to treat these bacteria. In 1995 drug-resistance was publicly acknowledged as a major health crisis in our society (Turkoski, 2005). Today this is an even bigger problem, not only are bacteria resistant but they are becoming multidrug resistant.

There are many reasons for bacterial resistance to antimicrobials. These reasons include the misuse of antimicrobials by professionals and patients, the increased use of broad-spectrum antimicrobials, the overuse in agriculture, and natural evolution of bacterium. Misuse of antimicrobials is said to be one of the primary reasons for rapid and widespread resistance (Turkoski, 2005). This can occur when doctors prescribe antimicrobials that are not needed for treatment of patients. Our society expects to be prescribed drugs for illness. Because of this, patients are sometimes given antimicrobials that are not effective against the disease or virus they have (Davies, 2004). Misuse can also be the fault of the patient. Either by forgetting to complete a prescription given by a doctor, or in order to save money a patient might save some of the antimicrobial in case they get sick again (Davies, 2004). Another big misuse of antimicrobials happens in agriculture. Many animals and plants are treated on a regular basis with antimicrobials to prevent disease or to enhance growth rates (Molt, 2005). Increased use of broad-spectrum antimicrobials is another reason that resistance has increased. This is also one of the leading causes for multidrug resistance. Broad-spectrum drugs target a wide range of organisms with the hope that one of the antimicrobials in the mixture will kill the infection (Turkoski, 2005). However this also exposes bacteria to a variety of other antimicrobials and if any bacterium does survive it could possibly be resistant to all the antimicrobials in the broad-spectrum drug, usually an entire class or family. The last reason that I will include is the natural evolution of bacterium. Bacteria can become naturally resistant to antimicrobials by horizontal gene transfer, the transfer of genetic material from one species to another, getting resistance genes from a bacterial species that has already become resistant (Brooker, 2009). This can happen in three main ways, conjugation, transduction, and transformation

(McDermott et.al., 2002). In some cases it can be incorporated into the bacterial chromosome by recombination (Blazquez, 2003). Conjugation is the transfer of genetic material between two bacteria that are physically connected by a pilus. Transduction is the transfer of genetic material from one bacterium to another through a virus. Transformation is when a bacterium picks up genetic material from the environment left there by a dead cell (Brooker, 2009).

Mechanisms of bacterial resistant are also important. Altered permeability to the antimicrobial is when it either cannot enter the cell or when the cell uses an efflux pump to eject it out of the cell. Inactivation of the antimicrobial means that the cell makes an enzyme that can disable it before it gets in place. Altered target site prevents the antimicrobial from recognizing the site it binds to so it cannot inhibit the bacterium. Finally a bacterium can change a pathway that is being blocked by an antimicrobial so it is rendered ineffective (Murray et al, 2007).

In 2007 Landon Snell isolated a strain of *E. coli* from a feedlot in McPherson Kansas that showed resistance to Tylosin (Snell, 2008). This is an antimicrobial that has been widely used as a feed additive by ranchers for promoting animal growth. It is also used by veterinarians against bacterial dysentery and respiratory diseases (Liu & Douthwaite, 2002). With multidrug resistance being a concern, I tested this Tylosin resistant *E. coli* against other antimicrobials to determine if the mechanism of resistance affected the susceptibility of this strain.

Tylosin is in the macrolide family. It inhibits protein synthesis at the 50S ribosomal subunit, by binding in the peptide exit tunnel (Lui & Douthwaite, 2002). Most of the antimicrobials that I chose to test this *E. coli* strain against are in different families that use different mechanisms to prevent bacterial growth. Amikacin is in the aminoglycoside family. They bind to the 30S ribosome and freeze the 30S initiation complex; they also cause the mRNA to be misread (Murray et al., 2007). Doxycycline is in the tetracycline family and also inhibits protein synthesis by binding to the acceptor site in the 70S ribosome and not allowing aminoacyl-t-RNA to bind there (Murray et al., 2007). Erythromycin, which, like Tylosin, is a macrolide, inhibits translocation of the peptidyl tRNA from the A to the P site on the ribosome by binding to the 50S ribosomal subunit of the bacterial 70S rRNA complex. This hinders the synthesis of proteins. Sulfamethoxazole with Trimethoprim is a sulfonamide and uses combination therapy. This combination blocks two, instead of one, steps in folic acid metabolism. This helps prevent the emergence of resistant strains (Murray et al., 2007). Penicillin is a beta-lactam, and is the last antimicrobial that I chose. It prevents certain steps from taking place in the synthesis of the cell wall in bacteria (McDermott et al., 2002).

My goal is to see if being resistant to Tylosin has any effect on the susceptibility of this *E. coli* strain to other antimicrobials. According to Molt, (2005) "…resistance to one kind of antibiotic often begets resistance to others.'"

MATERIALS AND METHODS

All experimentation was completed in the microbiology lab of McPherson College in McPherson, KS.

The bacterial strain used in this experiment was *E. coli* that showed resistance to Tylosin, it was isolated by Landon Snell in 2007 from a local feed lot, McPherson Co. Feeders, McPherson, KS. These bacteria were kept in Nutrient Broth in an incubator at 37C. This provided an environment in which the bacteria could continue to grow until they would be needed for further testing. The control bacterial strain used in this experiment was *E. coli* that is nonresistant to Tylosin. These bacteria were provided by Dr. Jonathan Frye.

There were five antimicrobials that were used in this experiment. These were in the form of saturated paper disks used for susceptibility testing called Sensi-Disc™. The first antimicrobial was Amikacin with a concentration of 30 µg. The second antimicrobial was Doxycycline with a concentration of 30 µg. The third antimicrobial was Erythromycin with a concentration of 15 µg. The fourth antimicrobial was Sulfamethoxazole with Trimethoprim having a concentration of 23.75 µg of Sulfamethoxazole and 1.25 µg of Trimethoprim. The Fifth and final antimicrobial was Penicillin with a concentration of 10 units.

The testing method used for the experiment was the Agar Disk Diffusion Method (Bopp, 1999). The manual for this method was followed with the alteration that a different control was used. The control strain I used was provided by Dr Frye and was nonresistant to Tylosin. Using the Agar Disk Diffusion Method I tested both Snell's Tylosin resistant experimental strain and the nonresistant control strain 16 times, for a total of 32 plates. Each of the 32 plates contained 5 Sensi-Discs, one for each of the antimicrobials that were tested. Figure 1.1 shows one of my experimental plates using the Agar Disk Diffusion Method, where you can clearly see the zones of inhibition. This is what a completed test should look like.

The data of this experiment was analyzed using the SigmaStat 3.5 program. The test used was an Unpaired t-test, however if the data were not normally distributed then a Mann-Whitney Rank Sum Test was used. The unpaired t-test was chosen because I wanted to compare the experimental groups from Snell's Tylosin resistant *E. coli* to the control groups of the non-resistant *E. coli*. Using the mean of each group I could determine if the

differences were statistically significant.

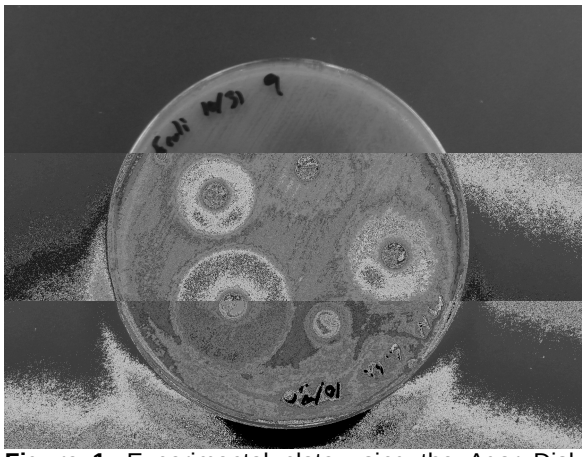

Figure 1. Experimental plate using the Agar Disk Diffusion Method. Notice the distinct rings of inhibition where the bacteria did not grow.

RESULTS

The Tylosin resistant *E. coli* showed a statistically significant difference when compared to the nonresistant *E. coli* to all five antimicrobials that it was tested with. Amikacin had a t-value of 11.50 and a P-value less than 0.001. Doxycycline had a t-value of 7.329 and a P-value of less than 0.001. Erythromycin failed the normality test but had a Mann-Whitney U Statistic of 88.0 and a P-value of 0.019. Penicillin had a t-value of 2.651 and a P-value of 0.017. There was a problem with my control group however so these results are not reliable. (Omitted from Figure 2). Sulfamethoxazole with Trimethoprim also failed the normality test but had a Mann-Whitney U statistic of 16.50 and a P-value of less than .001. These results are shown in Figure 2.

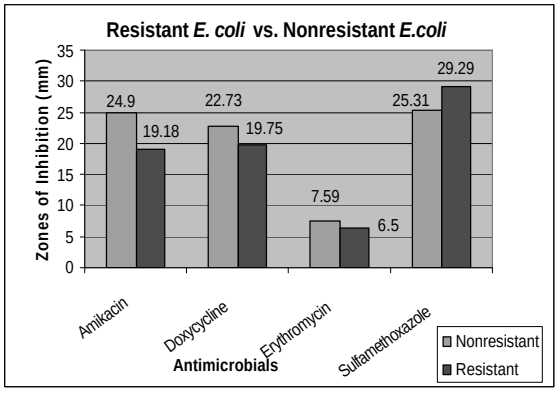

Figure 2. Resistant *E. coli* vs. Nonresistant *E. coli.* This table shows the mean of the resistant groups compared to the mean of the nonresistant groups.

DISCUSSION

The proposed question was whether the resistance to Tylosin made a difference to the sensitivity of the resistant *E. coli* to other antimicrobials. The results of this study show a statistically significant difference was found in all five antimicrobials. In Snell's Tylosin resistant strain was found to be more resistant, when compared to the nonresistant strain, to Amikacin, Doxycycline, Erythromycin, and Penicillin while less resistance was found to Sulfamethoxazole with Trimethoprim. However in the control group the zone of inhibition for Penicillin ran into zone of inhibition of Sulfamethoxazole with Trimethoprim. Because the zone of inhibition was so small for Penicillin it made measuring almost impossible. I was only able to measure, with any confidence, 3 plates. This didn't affect the measurements of Sulfamethoxazole with Trimethoprim because the zone of inhibition was so large that it could still be measured. Therefore, even though Snell's Tylosin resistant strain shows more resistance these results are unreliable.

The increased resistance is in line with the findings of Molt (2005) when she looked into the resistance caused by antimicrobials used as growth promoters in agriculture. She found that antimicrobials that were used as growth promoters in agriculture can cause bacteria to be resistant to many antimicrobials. These include antimicrobials in the same class, different classes, and sometimes even those in many different classes causing multidrug resistance.

The significance of this research reaches both the agricultural and medical fields. Resistance in humans can affect animals and resistance in animals can affect humans. "It is well established that bacteria, both resistant and susceptible, can be transferred from animals to humans and subsequently cause disease, so the use of antimicrobials in animals have some effect on human health." (Singer, 2005). If these bacteria do show resistance doctors will have a hard time curing the diseases they create, especially if the resistance affects the sensitivity to other forms or classes of antimicrobials.

For further research there are many more classes of antimicrobials that could be tested. I only tested only 5 of these classes. There are also other antimicrobials in each class that could be tested, including more in the macrolide family. Also the resistant bacteria were stored at 37C, would cooler or warmer storage temperatures affect resistance. Finally a lot of energy is used to produce enzymes or pumps for the bacteria to be resistant. Molt (2005) suggested that bacteria can sometimes revert back to the susceptible state if antimicrobials are not around for a while. Would it make a difference to the amount of resistance shown on how the bacteria are stored? The bacteria I tested were stored in a nutrient broth; would bacteria stored in a broth with

Tylosin be more resistant to other antimicrobials? Answering these questions would tell us more about this strain of resistant *E. coli*, and more about antimicrobial resistance.

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