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Isolation and Characterization of Antimicrobial Microorganisms in McPherson County Soil

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ABSTRACT

Since 2000, there have been only 20 new antibiotics that have rolled out to the public after FDA approval. However, bacteria are becoming more resistant to antibiotics that have already existed from the evolution of pathways to incur resistance. Feedlots and feedlot cattle are especially concerning to increase the number of resistant strains of bacteria as feedlots use a large amount of antibiotics to ensure growth of cattle. Tylosin is a very prominent antibiotic used in cattle feed, however much of the antibiotic is not absorbed and is left in the soil of the feedlot, where the natural microbes are adapting pathways to resist Tylosin effect. In this experiment, I attempt to find a type of microorganism that has developed antimicrobial pathways through adaptation to the new environment filled with Tylosin-resistant Bacteria. Potential candidates were harvested from feedlot soil samples and grown on Glycerol Yeast-Extract Agar plates, and applied to lab cultured *Escherichia coli* to test for antimicrobial activity.

Keywords: *antibiotic resistance, antimicrobial, Kirby-Bauer Testing Method, microorganisms*

INTRODUCTION

Ever since the discovery of penicillin, scientists have been on the search for new antibiotics. However, since 2000, only 20 new antibiotics have been released, 11 of which were naturally derived from plants, microorganisms, and other natural forms (Butler 2011). This can present a huge problem for mankind, because antibiotic resistant strains have become more and more prevalent over the past several years.

For half a century from 1940 to 1990, the typical response for the emerging resistance of antimicrobial resistant strains of bacteria was to discover a new type of antibiotic. However, in the more recent years, this has become a much less effective strategy, as more resistance has spread that new antibiotics can't combat (Livermore 2011).

There can be several causes to the emergence of antimicrobial resistance; however, there is one key factor that a majority of the scientific community agrees upon, which is the use and overuse of antibiotics. According to a paper by Levy (2002) "The emergence of MDR (Multidrug Resistance) is clearly related to the quantity of antibiotics and how they are being used." (Levy, 2002)

If this were to continue to occur, then eventually bacteria will become more resistant to current antibiotics (Borgman, et al. 2009). This is cause for an increased need in antibiotics. What can also be cause for alarm is that some of the new antibiotics might be close to useless. This is because some new forms of resistance are starting to emerge with tetracycline resistant bacteria (Peak et al. 2007). This should be cause enough to be on the search for new forms of antimicrobial agents.

According to Snell 2008, "Feedlots are especially concerning [regarding antibiotic resistance]." Cattle are given feed laced with antibiotics as well as antibiotic injections in order to keep infections to a minimum and optimize the growth of cattle and the amount of cattle able to be sold (Snell, 2008). One of the primary antibiotics given to cattle is Tylosin. Tylosin is in the class of antibiotics called Macrolides. Also included in this class are drugs like erythromycin and azithromycin. Tylosin inhibits bacterial protein production by way of inhibiting the 50S ribosome (Blackwood et al. 2008).

Gingerich (1977) showed that Tylosin was not absorbed into the muscles of cattle as well as previously thought. This could mean that much of the antibiotic is excreted through waste products and ends up in the soil where the cattle live and eat. With this fact known about the amount of antibiotics being given to cattle, one could also conclude that there would be several strains of Tylosin-resistant bacteria within the soil.

If the Tylosin is able to be absorbed into the soil present, then the natural microflora which inhabits the soil will be exposed to the antibiotic. This would lead to the killing off of the microflora that are not resistant to the antibiotic, leaving resistant strains of bacteria. Unless the various microflora have a positive symbiosis with one another, this would lead other microflora to adapt and produce some mechanism of killing off resistant microflora to survive and pass on their genetic traits. The purpose of this experiment is to see whether or not bacteria in the soil of feedlots have developed such a mechanism.

MATERIALS AND METHODS

When Fleming first discovered penicillin, he cut out

patches of the agar plates he used for growth of the mold which contains penicillin (Fleming 1929). This experiment did not contain the exact same methods used by him; however this experiment does hope to obtain similar results.

For this experiment, I obtained the samples of soil from the McPherson County feed yard and stored them in bags to keep outside bacteria and microbes out of the sample. The samples were obtained from either the surface or approximately 8 cm down from the surface. After the samples were obtained, the soil was dried at room temperature for approximately 1 week, and then approximately 1 gram of soil was weighed and mixed with 10 mL of distilled water.

Once the water was added, the solution was agitated vigorously, then applied to a Glycerol-Yeast Extract Agar (2.5 g Glycerol, Yeast-Extract 1 g, K_2HPO_4 0.05 g, Peptone 12.5 g, Agar 7.5 g, Distilled Water 500 mL) which was sterilized via Autoclave for 15 minutes at 121°C (Oskay, et al. 2004). The samples were grown for a period of 7-10 days on said plate, then colonies of the feedlot samples were transferred to a second Glycerol Yeast-Extract Agar plate using aseptic techniques, and were allowed to grow for another 7-10 days.

According to Snell 2008, a sample of bacteria was sent to an outside lab, where it was confirmed as being *E. coli*. Therefore, to test the antimicrobial activity in the feedlot samples*, E. coli* was selected to be grown on standard nutrient agar (Agar 1.5 g, Beef Extract 0.3 g, Peptone 0.5 g, Distilled Water 100 mL) which was sterilized by the same Autoclaving process as before.

Once the *E. coli* were cultured and the feedlot samples were grown and isolated, the *E. coli* were transferred to another standard nutrient agar plate. The feedlot samples were mixed in an aqueous solution, and said solution was applied directly to the plate of *E. coli* to simulate a Kirby Bauer Antimicrobial Susceptibility test. The test subjects were allowed to grow for 24 hours, then observed for determination of Antimicrobial Susceptibility.

RESULTS

Testing bacteria were able to be isolated from the feedlot in all samples collected, and grew on the Glycerol Yeast-Extract Agar as intended. Initially, the procedure of this experiment called for Tylosin-resistant *E. coli*; however, lab cultured *E. coli* proved to have no resistance to tylosin, and no resistant bacteria were able to be cultured, so the procedure was altered to include standard, non-resistant *E. coli*. After the *E. coli* was allowed to grow with the complimented feedlot test bacteria, there was a negative result for Antibiotic Susceptibility. There did not appear to be any inhibition of growth of *E. coli*.

DISCUSSION

The Purpose of this experiment was to explore the avenue of potential new forms of antibiotics located in areas where antibiotics are heavily used. This experiment was designed to show that over time, various strains of microorganisms have developed a method of defending themselves in order to pass on their genes to the next generation. However, the results of this experiment showed that there was no form of adapted antimicrobial properties.

One thing that could have potentially gone wrong is that I could have collected the soil samples at a time which is not suitable for growth. The samples were being collected in late January/early February, which is in the middle of Kansas winter. This year, temperatures have had highs averaged out around 5°C and have had low temperatures on average around -5°C. This could mean that any number of microorganisms could have died off before soil collection. This could easily be remedied by collecting samples during a time of major growth, namely between the months of March and October, possibly early November. This could provide other potential bacterial candidates that could be used for testing purposes.

More research is needed in order to explore new antibiotics in the world. Such studies could potentially look at where higher doses or more than one main type of antibiotics are used. This could lead to a higher probability of resistant bacteria of multiple species, which could increase the chance of other microorganisms to adapt to the new environment, and develop a mechanism for killing off rival bacteria.

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