Presence of Antibiotic Resistant *Escherichia coli* After Reduced Exposure to Antimicrobial Agents

Author: Ashlynn Vanatter
Faculty Mentor: Dr. Victoria Rhodes
Department: Missouri Southern State University Biology Department
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Introduction

Resistant bacteria pose a threat to society because the molecular mechanisms that cause antibiotic resistance are complex and can be hard to stop (Laxminarayan et al., 2013; Martens & Demain, 2017). Resistance to antibiotics is an issue that requires immediate attention because if healthcare systems want to continue to use antibiotics to save patients from severe infections, the mechanisms behind antibiotic resistance and what causes it must be understood (Richardson, 2017; Ventola, 2015).

The purpose of this study was to test if chronic low-grade usage of antibiotics promoted resistance over time. Using the theory of natural selection, which describes how populations change over time to alterations in the environment, and its relative contribution to the development of antibiotic resistance in bacteria, the relationship between dose over time of amoxicillin, ciprofloxacin, and trimethoprim and the amount of *E. coli* growth was examined.

Materials and Methods

To conduct this experiment, *E. coli* was inoculated into 100 mL of tryptic soy broth. After allowing the bacteria to grow, 10 µL of the *E. coli* broth was added to 21 microcentrifuge tubes. Each tube represented a single day in the course of each antibiotic.

After the bacteria was added to each tube, the process of introducing the antibiotics began. On the first day of the experiment, the low concentration of each antibiotic was added to all seven of their tubes. The next day, the low concentration antibiotic was added to six tubes, while the high concentration antibiotic was added to one tube. This process was continued until day seven, when all test tubes received the high dose of antibiotic. From this set up, a range of antibiotic exposure was achieved.

Before the introduction of the antibiotics was completed, however, working stock solutions needed to be made. To calculate the low and high concentrations needed, the working dose of each antibiotic was used. The working dose of each antibiotic was different, so preparation for each antibiotic varied.

In the second part of this experiment, a MIC assay was completed. For each antibiotic, 250 µL of the original high concentration antibiotic was added to two vertical wells. After the high concentration antibiotic was added, dilutions were performed. In the MIC assay, each well was diluted by a factor of one half. This was accomplished by pipetting 100 µL of plain TSB into the rest of the wells running horizontally. After this step, 100 µL of the high antibiotic solution was added into the next horizontal well. This process was continued until the last well was diluted. After this step, 16 µL of day one and day seven broth was placed in each well to examine the bacterial growth after 24 hours of incubation. The next day the bacterial concentrations in each well were recorded to process results for the experiment.

This experimental design was repeated three times to obtain more data and show a greater confidence in the results.

Results

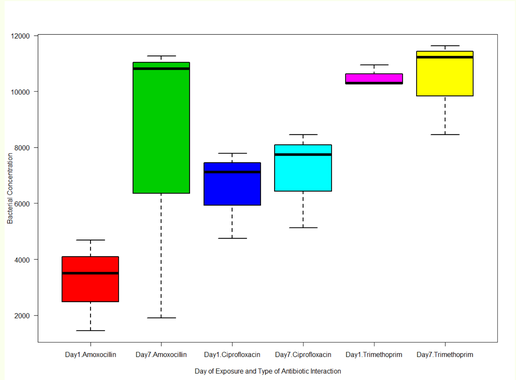

Figure 1

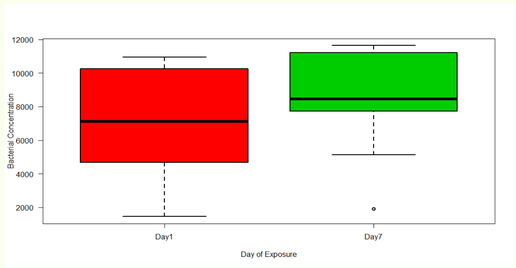

Figure 2

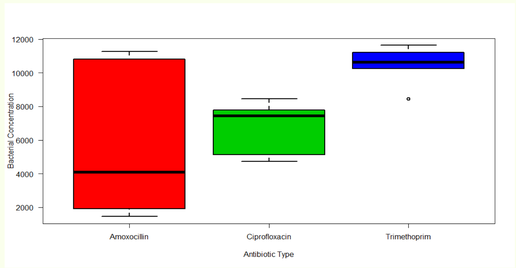

Figure 3

A 2-Factor ANOVA reported that in a full factorial model (Figure 1), there was not a significant interaction between antibiotic exposure and type on bacterial concentration ($F= 1.597$, $df=2,12$, $p= 0.2428$), therefore the interaction was removed. In a partial factorial model (Figure 2 and 3), there was not a significant difference between the bacterial concentration and the antibiotic exposure ($F=1.959$, $df=1,14$, $p=0.1834$). However, there was a significant difference between bacterial concentration and antibiotic type ($F=5.413$, $df=2,14$, $p=0.0181$).

Conclusions

The following null hypotheses were the testable expectations of the relationship between the concentration of antibiotic and bacterial concentration, and type of antibiotic and bacterial concentration.

Hypotheses

- $H_0$1: There is not a significant different between the concentration of antibiotic and bacterial concentration.
- $H_0$2: There is not a significant difference between the type of antibiotic and bacterial concentration.

After the partial factorial model was completed, the results rejected the first null hypothesis. There was not a significant difference between the bacterial concentration on day one and day seven across any of the antibiotics.

These insignificant results should not be misunderstood as the misuse of antibiotics not leading to higher bacterial levels or resistance. There are several reasons why significant results were not obtained between exposure in this experiment.

- Small concentrations
- *In vitro* study

The partial factorial model produced results that rejected the second null hypothesis. There was a significant different between the type of antibiotic and bacterial concentration.

- Future Research

References

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