Creating Antibiotic-Resistant E. Coli

**INTRODUCTION**

Bacteria are always evolving, especially bacteria we try to kill with antibiotics. Because of genetic variation that causes some bacteria to have different traits than others, not all bacteria are killable with commonly used antibiotics, such as ampicillin. When the majority of some bacteria is killed off, those few remaining that survived the antibiotic are free to reproduce to fill the decimated population. In the next application of antibiotic, the population is made up of entirely resistant bacteria, as chosen for by unintended artificial selection. Despite the dosage, time is all that keeps E. coli from growing when ampicillin is applied (Lawrence, Anthony). Our project aims to figure out what dosage of ampicillin creates the most anti-biotic resistant bacteria, and if any further exposures have any effect. Previous experiments have shown that antibiotic resistant bacteria is becoming more prevalent (Tadesse, et al.). With the knowledge of We think that all doses of ampicillin we apply will create a successful second generation population, but the highest dose will be the weakest and least populated.

**MATERIALS and METHODS**

For the project, we used:

* 2 petri dishes (plus one botched dish).
* 45 doses ampicillin (1microliter each).
* Cotton swabs (~6).
* E. coli.
* Sterile paper markers.

Subdivide the petri dishes into 6ths, numbered 0-5 on each. For dish one, do a wash of E. coli over the entire surface. Next, place paper markers in each section, and add a dose of ampicillin that corresponds to the section number (1 micro-liter for #1, 2mcl for #2, etc.). let grow for ~2 days (exact time forgotten), until bacteria growth is visible outside of the circles of effect for the antibiotics. Measure the circle of dead cells that surrounds the paper marker, and record with the corresponding dose. Swab from within the dead-zone from the antibiotic, and place in the spot for the corresponding dosage in the second petri dish. Ideally, antibiotic would be applied then, after an even distribution within the section from the swab. However, we let the bacteria grow to a certain population, then applied 5 mcl of ampicillin to all colonies. Due to the lack of visual difference between dead and living cells at the macro level, this was not the wisest.

**DATA/RESULTS**

The effect radii for ampicillin in the first round, given the dose and measured in centimeters:

Table 1. Title goes here

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Effect Radius of Ampicillin | | | | | | |
| Dose (micro-liter): | 0mcl | 1mcl | 2mcl | 3mcl | 4mcl | 5mcl |
| Effect Radius (cm): | 0cm | .7cm | .9cm | 1cm | 1.2cm | 1.4cm |

The effect radius is how far from the application of antibiotic bacteria does not grow. From this, we can judge the strength of the antibiotic applied. Graphed, you can see the gradual procession of potency:

Figure 1. Effect Radius of Ampicillin on E. coli

In the second generation of the experiment, where bacteria was swabbed from inside the radius effect, ampicillin should have been applied directly upon placement in the second petri dish. However, due to an error in which bacteria was allowed to grow for a while before the ampicillin was applied, measuring the effect radius of the second generation was impossible.

**CONCLUSION/DISCUSSION**

As it appeared (with the bacteria growing directly in the circle of application), there was no effect in the second application of ampicillin, despite 5 microliters applied to each section. As such, to compare the first generation with the second is meaningless, as the reading in the second generation is faulty. By comparing the effect radii of the first generation to zero, the effect noted in the second generation, we can be confident that the first generations effects are different (p =0.007). Had zero been the effect radius for all of the growths, we would see that antibiotic application of all quantities produces resistant bacteria. However, due to even the control “not being affected by ampicillin,” we can conclude the data meaningless.

A natural outcome, where the effects of the ampicillin were reduced (accurately) from the first generation to the second would show the adaptability of bacteria, and the dangers of using too many antibiotics. Artificial selection, coupled with transformation, can lead to diseases running out of control, because of the conditioning of bacteria for resistance and survival. Had this experiment functioned properly, it would have demonstrated that within two generations adaptations can become mainstream in populations. That data would support the hypothesis of growth after death, and probably the thoughts on higher concentrations being slow to regrow, yet stronger in the long run. However, due to errors, we cannot conclude this from this experiment. Applying the antibiotics in the second generation at the same time as the bacteria would allow the project to work as planned. A recommended next experiment would be this experiment, yet with those considerations applied.

**REFERENCES**

Lawrence Khadija, Anthony Michelle. *The Effects of Ampicillin on the Growth of Escherichia coli.* North Carolina State University Department of Microbiology. 2013. Web. May 4, 2014.

Tadesse DA, Zhao S, Tong E, Ayers S, Singh A, Bartholomew MJ, et al. *Antimicrobial Drug Resistance in Escherichia coli from Humans and Food Animals*. Center for Disease Control and Prevention, May 2012. Research Paper (Web). May 4, 2014.