Survival of the Fittest: Antibiotic Resistance in the Environment

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Some bacteria species can be used in a number of ways to benefit humans while other species can cause infection and disease. Infections caused by bacteria can be treated with antibiotics. Some bacteria have built defenses against antibiotics, creating a dilemma known as antibiotic resistance. In this study, the following objectives were investigated: 1) to determine the occurrence of antibiotic resistance in domestic and public environments; 2) to determine whether non-resistant bacteria can be made resistant by transformation.

To conduct this study, bacteria samples were collected from common surfaces in a home and in the Washington Metro Transit System. The samples were amplified and used to generate pure cultures. These cultures were subjected to a plasmid isolation process. To determine the occurrence of antibiotic resistance, the bacteria samples were tested against ampicillin, tetracycline, erythromycin, and clindamycin. Bacteria that are not resistant to the antibiotics were subjected to transformation (implantation with a plasmid carrying a gene for resistance). The transformed bacteria underwent another test against the antibiotics to see if the transformation process succeeded.

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Introduction

Antibiotic resistance is an ever-increasing problem in our world today. Overuse of antibiotics has resulted in stronger germs, which leaves us without cures for diseases that were once easily beaten. Commonly encountered bacteria such as *Staphylococcus* and *Salmonella* are becoming resistant to widely prescribed antibiotics such as penicillin and erythromycin.

The primary purpose of this project was to find the incidence of antibiotic resistance in bacteria found in domestic and public environments by collecting bacterial samples from both areas and testing them against the commonly prescribed antibiotics. These antibiotics are penicillin, tetracycline, clindamycin, and erythromycin. Bacteria that prove to be non-resistant will be implanted with a plasmid carrying a gene for resistance through transformation.

Methods

Bacteria Collection and Amplification

This exploration of antibiotic resistance began by securing bacteria samples from ten different locations, five from a domestic environment and five from the Washington DC Metro Transit System. The domestic samples came from the following places: bathroom faucet, kitchen sink, stove, toilet seat, and keyboard. The public environment samples came from the Washington DC Metro Transit system: handrail, armrest, escalator rail, window, pay phone.

These samples were obtained by swabbing each location with a single sterile cotton swab. The swabs were left in separate tubes containing five milliliters of Luria broth for forty-eight hours. Following this growth period, the samples were put in an incubator overnight at 37°C with shaking.

Next, the samples were removed from the shaking incubator and ten petri dishes containing LB agar were each streaked with a bacteria sample. The dishes were then inverted and placed in an incubator for twenty-four hours at 37°C.

After the 24-hour growth period, single colonies from each dish were placed in separate test tubes. On one plate (sample 6), there were two distinct colonies growing. Those colonies were grown in separate tubes of Luria broth and this process brought the total of bacterial samples from ten to eleven. The eleven test tubes were placed in an incubator overnight at 37° C with shaking.

When the colonies were removed from the shaking incubator, the process of streaking petri dishes and incubating them was repeated. The samples were then taken and placed in separate test tubes containing ten milliliters of Luria broth. Instead of putting the test tubes in the shaking incubator again, they were left at room temperature.

Antibiotic Sensitivity Testing

The four antibiotics chosen for this investigation were penicillin, tetracycline, clindamycin, and erythromycin. To test these four antibiotics, each bacteria sample was treated with each drug and placed in an incubator. After the discs were removed from the incubator, the zones of inhibition around each antibiotic disk were measured and recorded.

Plasmid DNA Isolation

Plasmids, which are self-replicating circular DNA, were extracted from each

bacterial culture by using the Plasmid Extraction Spin Miniprep Kit Protocol by Qiagen. The protocol was designed for purification of up to 20 μg of high-copy plasmid DNA from 1–5 ml overnight cultures in LB (Luria Bertani) medium.

Restriction Enzyme Digestion

Restriction enzyme digestion is a process by which DNA is digested into smaller pieces due to the recognition of "cut-sites" by the enzyme. "Cut-sites" are specific DNA sequences at which DNA is cut by restriction enzymes that are also known as endonucleases. First, two restriction enzymes and a buffer were chosen. In this case, those were BamH I, Hindi III, and Buffer E. Samples 1 through 6b were treated with BamH I and Samples 7 through 10 were treated with Hindi III. After adding the digestion ingredients (the enzyme and buffer) to eppendories the solution was lightly flicked in order to ensure that the enzyme mixed thoroughly. The solution was then centrifuged at maximum speed for 20 seconds. Subsequently, it was incubated in a water bath at 37°C for two hours. After being removed from the water bath, 1.7 μl of 6x dye, 6 μl of DNA, and 2.3 μl of de-ionized water (dH20) were added to each eppendory. To view digested DNA made by the restriction enzymes, the solutions were run on a 2% Agarose gel and visualized using ethidium bromide and UV light.

Transformation

Transformation is the genetic change of a bacterium after exposure to and recombination with isolated DNA from a genetically different bacterium. Plasmids carrying genes for resistance to tetracycline were chosen for the transformation process. Following a series of steps including centrifuge, re-suspension, and incubation, the samples were heat shocked at 42°C for exactly 2 minutes and then allowed to cool to room temperature. Each sample was spread on LB agar plates and treated with one disk containing 30mg of tetracycline. The plates were then inverted and placed in an incubator at 37°C overnight.

CONCLUSION

Because of penicillin's widespread use since the 1930s, most known strains of bacteria have developed full or partial resistance to the drug. That fact most likely explains the relatively low performance of penicillin throughout each trial. Tetracycline's high rate of performance in this experiment is due to the dosage which was used (30 mg) because much smaller amounts of the drug can completely inhibit the growth of bacteria. For example, only 1.17 μg of tetracycline is needed to totally restrain the growth of *E. coli.* Clindamycin was most effective against Samples 3 and 7, which were both gram-positive cocci. Erythromycin's performance in this investigation was similar to that of clindamycin and above that of penicillin, most likely because of their similar spectrum.

This investigation did not show a very high incidence of antibiotic resistance in our environment as hypothesized; however, the threat of antibiotic resistance has not lessened. As our world becomes smaller and more susceptible to the rapid spread of infectious diseases, it becomes more important to emphasize the correct use of antibiotics.