

Spectrophotometer Measurements of Bacterial Colonies Exhibiting Increasing Antibiotic Resistance

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Abstract. As the use of antibiotics and antibacterial solutions continues to increase, the resistance to these drugs continues to increase as well. During this experiment bacteria were exposed to the antibacterial solution; Lysol® (Reckitt Benckiser Inc., Parsippany, NJ 07054). Readings were taken before and after the initial exposure to the diluted Lysol solution. The re-growth to the bacteria was monitored as well. During the experiment, the bacteria were placed in a dilution of 9mL of broth and 1mL of bacteria and a reading was taken using a spectrophotometer which measures density in a solution. Then a dilution of 8mL of broth, 1mL of bacteria, and 1mL of Lysol was made and readings were taken. The same bacteria were re-grown in a dilution of 10mL to 1mL and the process was repeated. The bacteria used during this experiment implied an increase in resistance after the second exposure to the Lysol solution. After the first exposure there was a greater decrease in bacteria cells present than after the second exposure possibly implying an increased immunity to the Lysol.

Keywords: Microbiology, Bacteriology, Serrita, Antibacterial Resistance

INTRODUCTION

The re-growth of bacteria exposed to an antibacterial solution, is possibly shows a bacteria colony's increasing resistance to an antibacterial solution, or antibiotic over time. As bacteria encounter an antibiotic for the first time, most of the bacteria are killed, but the few remaining are too small to cause harm, yet are able to re-grow in a suitable environment. The second time these bacteria encounter the antibiotic; many are killed, but slightly more survive and re-grow. The third time these bacteria are exposed to the antibiotic less are killed and the rest re-grow. This process occurs until the bacteria obtain complete resistance to the antibiotic through genes developed. In the plasmid of the bacteria, genetic material has a code within it which allows the bacteria to become gram-positive (without resistance) to gram-negative (with resistance). As bacteria increase their resistance to the antibiotics, it will cause problems to occur. Bacteria which previously caused sickness once were able to be killed within a week. But the over use of these drugs are beginning to back fire and bacteria are becoming immune to the drugs and are coming back stronger and stronger. The medical community is plagued by this and the demand for new antibiotic drugs is high. Bacteria with resistance have the ability to reproduce creating more antibiotic resistance bacteria. Many scientists are researching this resistance and also researching in the field of genetic engineering. When scientists obtain the full understanding of genetics, especially within bacteria, it will allow humans to put an end to certain diseases and sicknesses which have plagued the world for hundreds of years. But until then, bacteria will become increasingly resistant to antibiotics. There are numerous ways the count bacteria. A scientist can use a grid to count the amount of colonies per square inch, square centimeter, etc. Another way the measure the amount of bacteria is using a spectrometer to measure the density of a grouping of cells in a solution by sending a beam of light through the solution and measuring the wavelengths.

This experiment concerns bacteria resistance to antibacterial solutions. The experiment focuses on the increasing resistance to antibiotics over time. As bacteria are exposed to an antibacterial solution such as Lysol® they become less prone to being destroyed by the solution. In order for this experiment to show the bacteria's resistance it is necessary to take readings with bacteria before and after its exposure to the antibacterial solution. The way to take these readings is by measuring the density of the cells needed to measure in a spectrophotometer. The number the spectrophotometer reads measures density, the lower the

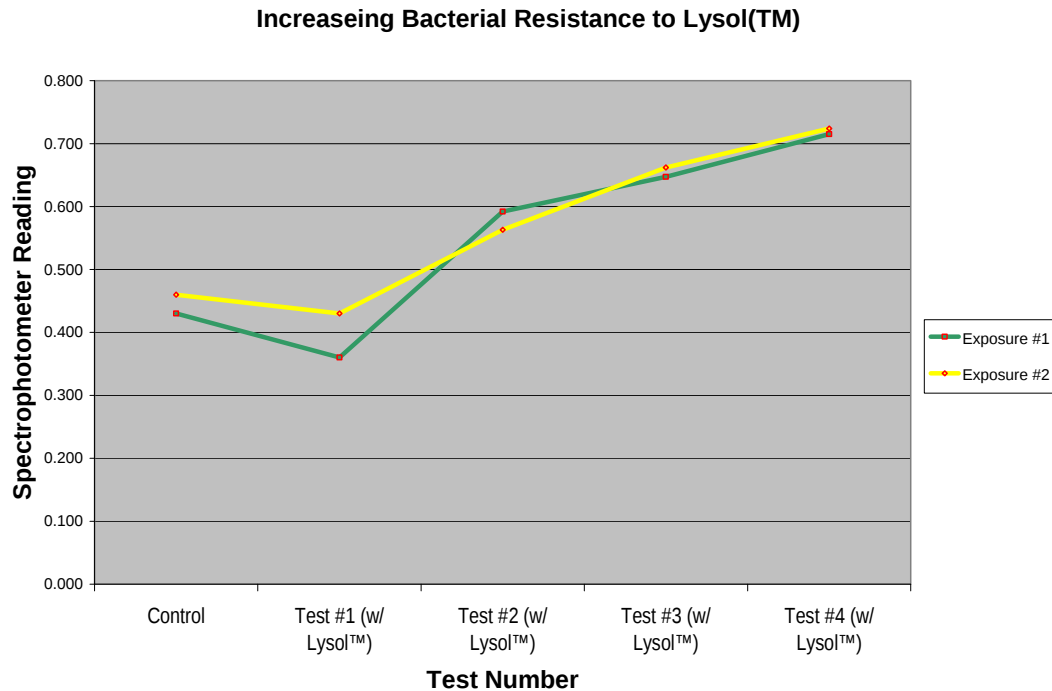
number, for example .290, the less cells are in the sample being read, the greater the number, for example .706, the more cells in the sample. In order for a spectrophotometer to measure density, it sends a beam of light through the solution and measures the wavelengths of the light passing through the solution. The first time the bacteria is grown and exposed to the Lysol solution, many bacteria was be killed. After they are grown a second time and exposed again to Lysol again, fewer bacteria was die. This experiment may then indicate the bacteria increasing their resistance to the Lysol solution.

METHODS

The bacteria strain of Serrita was grown in broth. Then 15mL test tubes were used to hold dilutions of the bacteria cells. The first dilution created was the control; which was 1mL of bacteria cells and 9mL of broth. Then, a reading was taken using a spectrophotometer, which measure the density of a solution by sending a beam of light 600nm wide through the solution and then measures the wavelengths that go through the solution. Following this reading, another dilution was made; this dilution would contain the first generation of cells exposed to a dilution form of Lysol (the dilution form of Lysol is 1mL to 100mL of water). The first generation of bacteria cells was in the original dilution of 1mL of bacteria cells, 1mL of Lysol solution, and 8mL of broth. Both dilutions; the control and the first generation exposed to Lysol, must have the same volume of 10mL in order to obtain an accurate reading. Then using a spectrophotometer the solution was being tested and the decrease in density of the solution, which translates into a decrease in bacteria cells, was being recorded. Following this, 1mL of these bacteria cells exposed to the Lysol solution was be re-grown in 9mL of broth, this dilution was become then control of the second generation of bacteria and a reading is taken. Now, the dilution for the second generation of bacteria cells exposed to the Lysol solution was be created. This dilution consists of 1mL of bacteria cells (already exposed to Lysol), another 1mL of the Lysol solution, and 8mL of broth. The decrease in density was then being noted. During the second exposure the decrease in density was be less then the decrease in density of the first exposure, which means less bacteria cells were killed after the second exposure to Lysol then after the first exposure to Lysol.

RESULTS

After growing the bacteria and putting it into a dilution of 1mL of bacteria cells into 9mL of broth. Then using a spectrophotometer a control reading was taken, Figure 1 shows the reading of .430 was noted. Following this 1mL of Lysol, was added to a dilution of 1mL of bacteria cells and 8mL of broth. After this exposure to Lysol, a reading of .360 was noted. The re-growth of the bacteria was noted as well, the readings were .592, .647, and .715. These same bacteria were put into a dilution of 1mL of bacteria cells and 9mL of broth and a control reading of .460 was noted. Then 1mL of bacteria cells previously exposed to Lysol was added to a dilution of 1mL of Lysol and 8mL of broth. The reading noted was .430. The re-growth readings were .563, .662, and .724.



[Figure 1] - This is a graph exhibiting the possible bacteria resistance to Lysol over two generations, through examining increases in a density of the bacteria solutions.

DISCUSSION

Figure 1 Exhibits bacteria gaining a possible resistance to Lysol, fewer bacteria are killed. During this experiment a larger number of bacteria were killed when first exposed to the Lysol solution then after the second time the bacteria were exposed to the Lysol solution. The control reading of the first generation of bacteria was .430 after exposed to the Lysol solution a reading of .360 was taken. That is a .090 drop in density of the bacteria solution. The control reading of the second generation, which was re-grown bacteria from the first generation, was .460, after being exposed to the Lysol solution a reading of .430 was taken. This is only a .030 drop in density [Figure 1].

CONCLUSION

This many indicate less bacteria were killed after they were exposed to the Lysol solution a second time. These bacteria may now represent a low level of resistance to Lysol.

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